BOOK 12

Chrom-Ed Book Series

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PREPARATIVE CHROMATOGRAPHY

Chrom-Ed Book Series

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Introduction

Preparative chromatography can be a very ambiguous term and its meaning will often depend on the *raison d'être* for its use. To the forensic chemist, preparative chromatography may mean the isolation of only a few microgram of material for structure elucidation by subsequent spectroscopic examination. To the biochemist, it may mean the isolation of a few milligrams of a substance required for assessing its physiological activity. In contrast, to the organic chemist, preparative chromatography will often mean the isolation of 5 or perhaps even 50 g or more of a pure intermediate for subsequent synthetic work (this can be particularly important in the separation of chiral mixtures). Thus, the amount of material that is separated does not necessarily determine whether the separation can be classed as preparative or not. However, all preparative separations involve the *actual collection of an eluted component* and does not merely comprise peak profile monitoring for quantitative estimation and elution time measurement. It is interesting to note that the technique of chromatography, originally invented by Tswett in the latter part of the nineteenth century, was not initially developed for analytical purposes, but for the isolation of some specific pigments from plant extracts. In fact, all the early applications of chromatograph were exclusively for preparative purposes and it was not until gas chromatography (GC) was introduced by Martin and Synge (1) was the technique used for analytical purposes. Even after the introduction of GC, liquid chromatography (then called *column chromatography*) was still used largely for preparative work. Liquid column chromatography evolved from a preparative procedure into an analytical technique during the late nineteen sixties, largely provoked by the development of *high*

performance liquid chromatography (HPLC), which, in turn, was largely sparked off by the successful development of GC. Initially, column loads were increased for preparative purposes by increasing the dimensions of the column both in GC and in HPLC. However, this approach has distinct limitations.

If the column radius is increased, unless special packing techniques are employed, the packing procedure becomes inefficient and the packing itself unstable. In addition to maintain the optimum mobile phase velocity, the flow rate will need to be substantially increased and the consumption of mobile phase will eventually become economically impractical. Conversely, if the column length is increased, then the impedance to flow will become greater leading to high column pressures. If large column radii are employed, then the mechanical strength of the column system will limit the maximum permissible pressure. Consequently, lengthening the column will eventually require the particle diameter to be increased to provide adequate permeability. Increased particle diameter will, in turn, reduce the column efficiency, which may impair the resolution of the compounds of interest. It is seen that scaling up a chromatographic separation can be quite complex as many of the controlling factors are interacting and so the optimum parameters required by the separation are not easy to define. To understand the problems associated with preparative chromatography the factors controlling the *loading capacity* of a column need to identified.

The Loading Capacity of a Column

The column system that is most commonly employed in preparative chromatography is the *axial flow cylindrical column*. Other column systems that are used very effectively in preparative chromatography are *radial flow* columns, and *simulated moving bed columns*, but these systems are more complex and require very special and expensive apparatus. In addition, initial work with traditional columns will usually be necessary in order to evaluate the separation problem and thus, justify the necessary expense that is involved with these more sophisticated type of column systems.

It has been shown (see book 6 in this series), that the maximum sample feed volume (V_i) or sample mass (M_i) that can be placed on a cylindrical column is directly proportional to the plate volume of the column:

$$M_i = A_m\sqrt{n}\left(v_m + Kv_s\right)$$

or:
$$V_i = A_v\sqrt{n}\left(v_m + Kv_s\right)$$

where K is the distribution coefficient of the solute between the two phases,
(v_s) is the volume of stationary phase per plate,
(v_m) is the volume of mobile phase per plate,
(n) is the column efficiency,
(A_v) is a constant for sample volume,
and (A_m) is a constant for sample mass.

Now:

$$V_i = A_v\sqrt{n}\left(v_m + Kv_s\right)$$
$$= A_v\sqrt{n}\, v_m(1+k)$$

From the Plate Theory (see book 6 of this series), $V_m = nv_m$, where (V_m) is the total volume of mobile phase in the column or the column dead volume.

Thus:
$$V_i = A_v\frac{V_m}{\sqrt{n}}(1+k)$$

Now:
$$V_m = \square\square r^2 L$$

where (r) is the radius of the column,
(L) is the length of the column,
($\square$) is the fraction of the column containing solvent.

Thus:
$$V_i = A_v\frac{\square\square r^2 L}{\sqrt{n}}(1+k)$$

Now (e) can be taken as approximately 0.6:

Thus, $$V_i = A_v \frac{1.9 r^2 L}{\sqrt{n}}(1+k)$$

and: $$M_i = A_m \frac{1.9 r^2 L}{\sqrt{n}}(1+k) \qquad (1)$$

The magnitude of (A_V) will be determined by the nature of the separation, and (A_m) will be defined by the loading capacity of the stationary phase and, to some extent, by the shape of the adsorption isotherm.

Equation (1), although, apparently simple in form, has some very significant implications on preparative column design. It is clear that increasing radius and length of the column increases both the maximum sample volume and the maximum sample mass. It is also seen that increasing the column length will also increase the column efficiency (unless it is accompanied by an corresponding increase in the particle diameter).

However, increasing the column efficiency will have the opposite effect, as seen by equation (1), it will *reduce* the maximum sample load. Consequently, if the necessary efficiency to achieve the required separation has been obtained, then if the column is lengthened to increase the loading capacity for optimum performance, either the flow rate will need to be increased to reduce the efficiency and thus maintain the maximum loading, or the particle size will need to be increased to reduce the efficiency to its required value. However, an increased flow rate will also reduce separation time and thus increase sample throughput. Conversely, the alternative use of larger particles will increase column permeability and thus the column can be operated at a lower pressure and be constructed of lighter materials. Again, a an alternative, if the sample is merely a two component mixtures such as a chiral pair, using automated sampling on a long permeable column will permit multiple injections, so that there can be a number of solute pairs in the column being separated at one time and be eluted sequentially, each solute being eluted alternately with the other. Such a system will also help increase throughput and thus, improve productivity.

One of the limiting factors that controls the throughput of the preparative chromatograph will be the maximum permissible sample volume. The maximum sample volume is that volume that will limit any loss of resolution to an acceptable and pre-defined level. To extend the basic examination of those factors that control the efficient operation of preparative columns, the factors that determine the maximum sample volume will now be considered in detail. To do this, an equation will be derived that allows the maximum sample volume to be calculated.

The Maximum Sample Volume

Any finite volume of sample placed on to a chromatography column will have an intrinsic band variance, and this variance will be added to the variance from the usual dispersion processes that take place in the column to provide a value for the ultimate peak variance. Consequently, if the column efficiency is not to be seriously reduced the maximum volume of sample that can be placed on the column must be limited.

Consider a volume (V_i) of sample, injected onto a column. This sample volume will constitute a rectangular distribution on the front of the column. Now, (as discussed in book 9 of this series) the variance of the peak eluted from the column will be the sum of the variances of the injected sample plus the normal variance of the eluted peak.

Thus: $$\sigma^2 = \sigma_i^2 + \sigma_c^2$$

where σ^2 is the variance of the eluted peak,
σ_i^2 is the variance of the eluted sample,
and σ_c^2 is the variance due to column dispersion.

The maximum increase in band width that can be accepted due to any (and all) extraneous dispersion process is clearly optional, and will be determined by the character of the separation, and the purity of the required product. Klinkenberg (2) suggested a 5% increase in standard deviation (or, *ca*. a 10% increase in peak variance) was the maximum extra-column dispersion that could be tolerated without serious loss in

resolution. This criteria is the generally accepted standard in analytical LC.

The variance of a rectangular distribution of sample at the front of a column is given by $\frac{V_i^2}{12}$.

Assuming (according to Klinkenberg) the peak width is increased by 5% due to effect of the sample volume, then:

$$\frac{V_i^2}{12} + \left(\sqrt{n}\left(v_m + Kv_s\right)\right)^2 = \left(1.05\sqrt{n}\left(v_m + Kv_s\right)\right)^2$$

Consequently,

$$\frac{V_i^2}{12} = n\left(v_m + Kv_s\right)^2\left(1.05^2 - 1\right)$$

$$= n\left(v_m + Kv_s\right)^2 0.102$$

Thus,

$$V_i^2 = n\left(v_m + Kv_s\right)^2 1.23$$

$$V_i = \sqrt{n}\left(v_m + Kv_s\right) 1.1$$

Bearing in mind from the Plate Theory (see book 6 of this series) that:

$$V_r = n\left(v_m + Kv_s\right)$$

Then:

$$V_i = \frac{1.1V_r}{\sqrt{n}}$$

The equation indicates that the maximum acceptable sample volume can be calculated from the retention volume of the solute concerned and the efficiency of the column. It is also shows, that the maximum sample volume will increase with the retention volume of the solute, and decrease inversely as the square root of the efficiency.

From the point of view of preparative separations the practical implication is that the *sample volume* can be *increased* by adjusting the mobile phase so that the solute pair is eluted later. Apparently, this procedure would also increase the separation time, but this may not always be a disadvantage as for simple separation this may allow more

than one sample to be separated in the column at one time. The maximum sample volume is important when the separation ratio of a solute pair is relatively small, and adequate resolution is only just obtainable from the column. If, however, the stationary phase provides good selectivity between the solute pair of interest, and provides a length of base line between the two peaks, then column overload might be justified.

Sample Volume Overload

Consider the separation depicted in figure 1 (the retention parameters are labeled according to the plate theory as discussed in Book 6). Examination of the figure shows that the column could be heavily overloaded, to allow the peaks to spread until they touched at the base, before resolution would be lost. Under these conditions the principle of the summation of variances cannot be used, as when the sample volume becomes excessive, the dispersion of the peak becomes, to the first approximation, equal the volume of the sample itself. Presented in a different way, the sample volume acts as a part of the mobile phase and contributes to the elution process in the same manner. Consequently, referring to figure 1, the peak separation in milliliters of mobile phase will be equivalent to the sample volume plus the sum of half the base widths of the respective peaks. Bearing in mind that half the peak width is equivalent to two standard deviations, then:

$$(\alpha - 1)\, n\, K_A v_s = V_L + 2\sqrt{n}\left(v_m + K_A v_s\right) + 2\sqrt{n}\left(v_m + K_A v_s\right)$$

(K_A) and (K_B) are the distribution coefficients of solutes (A) and (B) and (α) is the separation ratio of solutes B and A respectively.

Rearranging:

$$V_L = (\alpha - 1)\, n\, K_A v_s + 2\sqrt{n}\left(\left(v_m + K_A v_s\right) + \left(v_m + K_A v_s\right)\right)$$

Noting that (see book 6):

$$nK_A v_s = V'_A,\ nK_B v_s = V'_B,\ \frac{V'_A}{V_o} = k_A,\ \frac{V'_B}{V_o} = k_B,\ k'_B = \alpha\, k'_A$$

and $V_o = nv_m$

$$V_L = V_o\left[(\alpha - 1)k'_A - \frac{2}{\sqrt{n}}(2 + k'_A + \alpha k'_A)\right] \qquad (2)$$

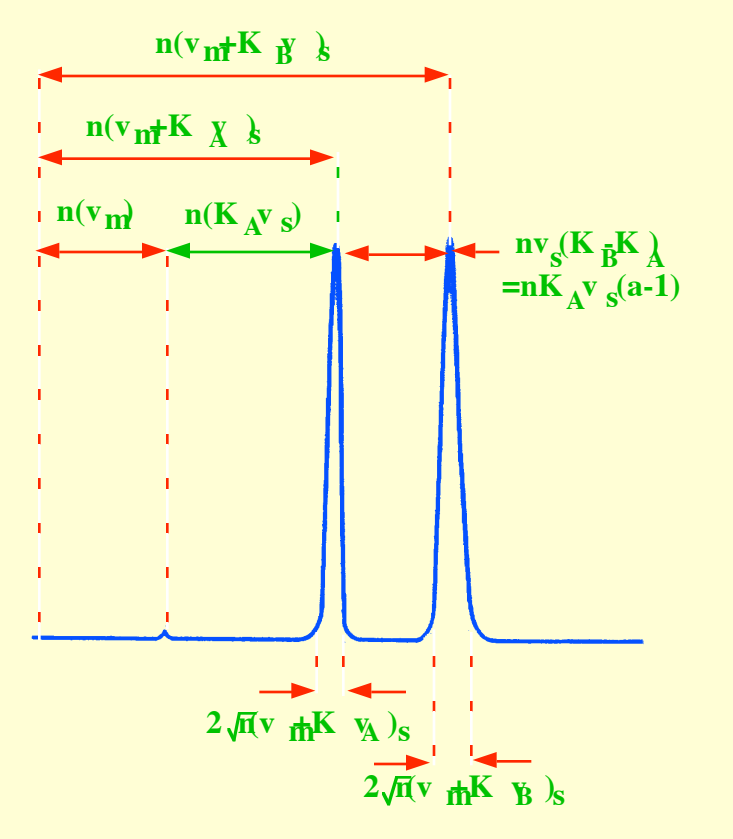


Figure 1 Theory of Volume Overload

Equation (2) can be used to calculate the maximum sample volume that can be placed on a given column for a range of solutes eluted at different capacity ratios and having different separation ratios.

The chosen column was assumed to be 40 cm long, 2 cm I.D., having a dead volume of 75.4 ml ($\pi r^2 l$). The maximum sample volume was calculated for a range of separation ratio values (α) and capacity ratio values (k) for the first eluted peak. The results are shown in figure 2. It is seen that the maximum sample volume can vary over a very wide range of values. Employing the defined column packed with 20 μm particles,

and operated at its optimum velocity to give 12,500 theoretical plates, if the separation ratio is as small as 1.5, and the first solute is eluted at a (k') value of 2.0, a volume as large as 66 ml can be still placed on the column.

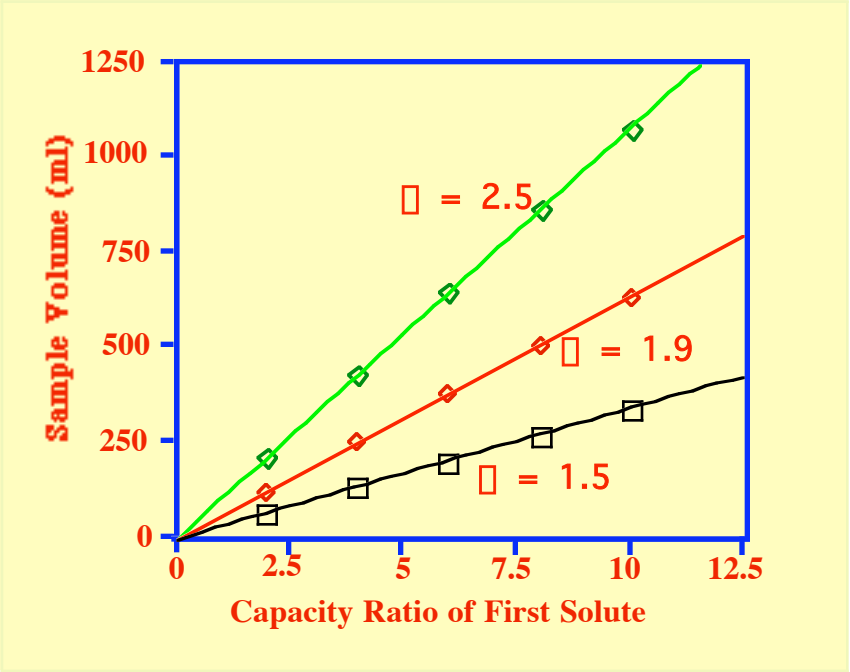
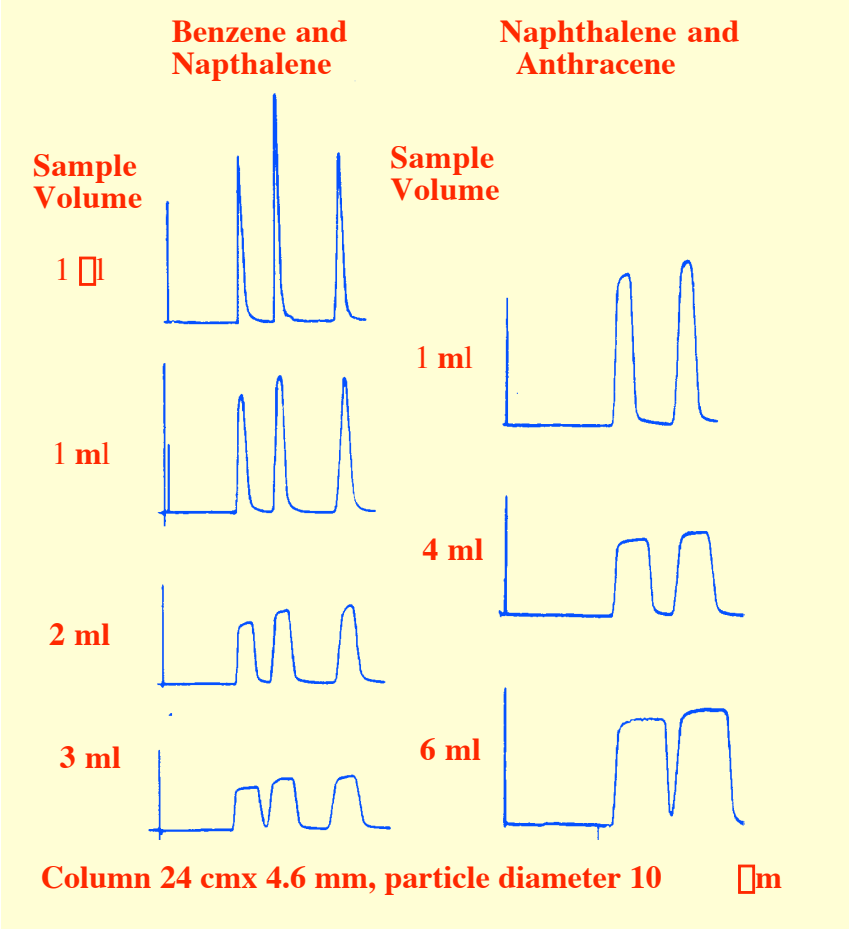


Figure 2 Graph of Maximum Sample Volume against (k') for the First Solute

At the other extreme if the first solute is eluted at a (k') value of 10 and the separation ratio is 2.5 then the sample volume can be over 1 liter. Nevertheless, it should be emphasized that the mathematical argument tacitly assumes that sample is injected onto the column as a *solution, in the mobile phase*. (*i.e.* the sample solvent does not change the elution conditions in any way). In addition, it also assumes that the solute concentration in the sample solution is not strong enough to produce significant *solute/solute interaction* in the mobile phase and consequently, also effect the conditions of elution.

The effect of volume overload on the elution profiles of solutes separated on an LC column was examined by Scott and Kucera (3) and the results they obtained are shown as elution curves in figure 3. The column dead volume was assumed to be *ca* 2.5 ml. The chromatographic properties of

the three solutes chromatographed on the column at a flow rate of 1 ml/min. together with the respective efficiencies are shown in table 1.



After, J. Chromatogr., Ref. [3]

Figure 3 An Experimental Example of Volume Overload

It is seen that the column was operated well above its optimum flow rate so the maximum efficiencies obtainable for each solute were not realized.

Table 1 Chromatographic Properties of the Three Solutes Separated on the Column Used for Overload Experiments

	Benzene	Naphthalene	Anthracene
Capacity Ratio **k**	1.18	2.33	4.31
Efficiency **n**	1850	4480	5470
Retention Ratio α	—	1.97	1.85
Sample Volume V_L	3.1 ml	6.1 ml	—

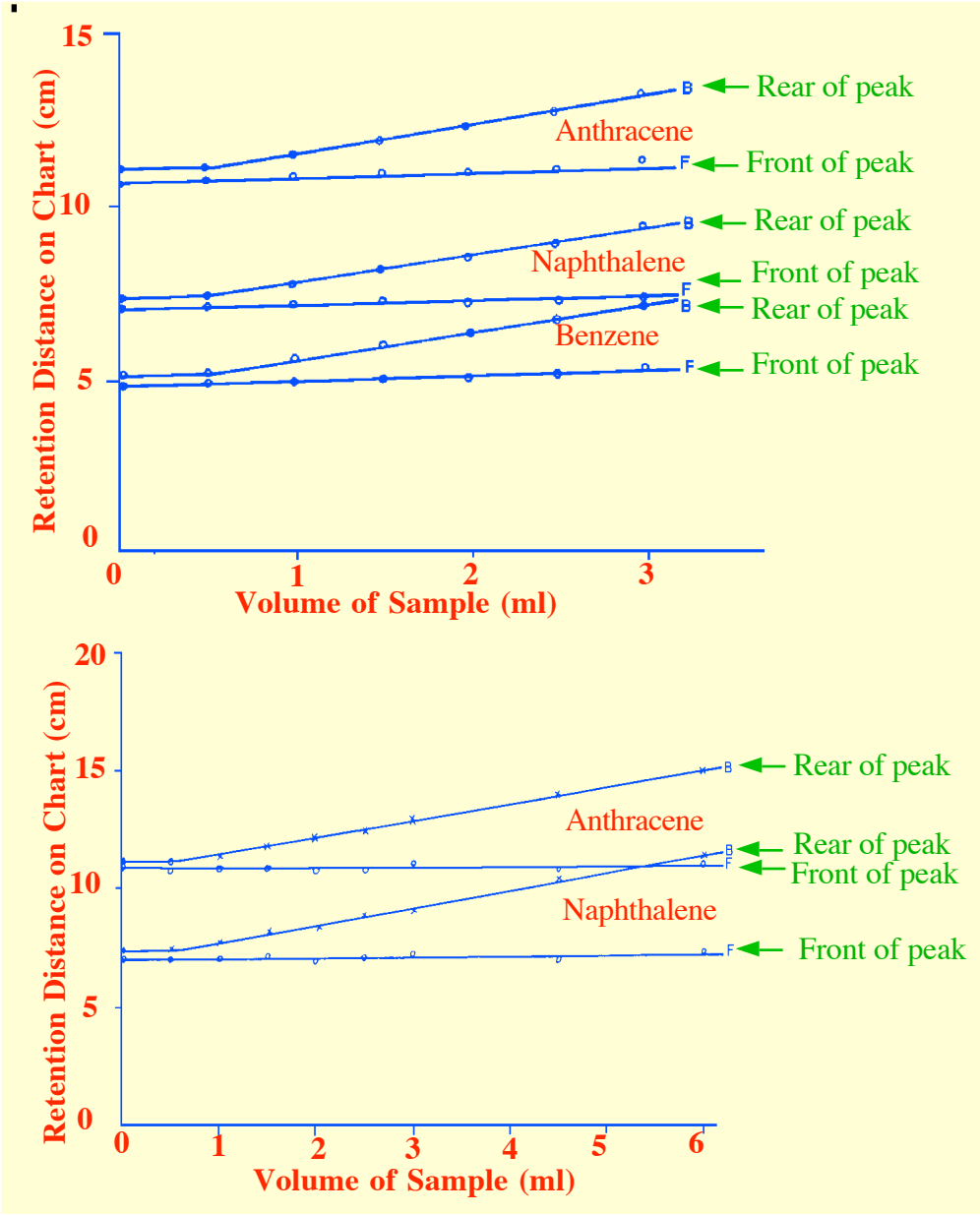
It also appears that the dispersion that occurs in the sample loop from Newtonian Flow (see book 9 in this series) seriously reduces the efficiency of the two first peaks and, consequently, it is not until the capacity ratio of the solute reaches 4, or more, could reasonable efficiencies be obtained. Notwithstanding, the results unambiguously demonstrate the advantages of column overload.

In the chromatograms on the left hand side of figure 3, 10 □l, 1 ml, 2 ml, and 3 ml of sample was placed on the column each containing 176 □g of benzene, 9 □g of naphthalene and 0.3 □g of anthracene. So all the samples contained the same mass of each solute. It is seen that a sample volume of 3 ml (theoretical value calculated from equation (2), was 3.1 ml) just allows benzene and naphthalene to be separated.

Similarly, in the chromatograms shown on the right hand side of figure 3, 2, 4 and 6 ml of a solution each containing 9.0 mg of naphthalene was injected onto the column and. The maximum sample volume that just permits the separation of naphthalene and anthracene, identified experimentally was 6 ml (theoretical value calculated from equation 2 was 6.1 ml). Destefano and Beachel (4) has also investigated the effect of volume and mass overload on resolution. They concluded that, given the choice, it is advantageous to overload a column with a *large volume of a dilute solution* of sample, as an alternative to using a *small volume containing a high concentration* of sample. They reported, however, that the validity of this conclusion, appeared to depend somewhat on the capacity ratios of the eluting solutes. In figure 4 the retention distance (measured in cm along the chart) is plotted against the sample volume

It is seen from figure 4 that the peak front of each solute has a constant retention irrespective of the volume of charge. The back of the peak, however, only maintains a constant retention distance up to a sample volume of 0.5 ml for the three component mixtures, and up to 1 ml for the two component mixture. Subsequent to these limiting sample volume values, the retention of the back of the peak appears to increase linearly with charge volume. It is also interesting to note that peak dispersion is

the same for each solute and is independent of the nature of the solute or its capacity ratio (k'). The peak dispersion towards greater retention is characteristic of volume overload which, as will be seen below, will not be true for mass overload.

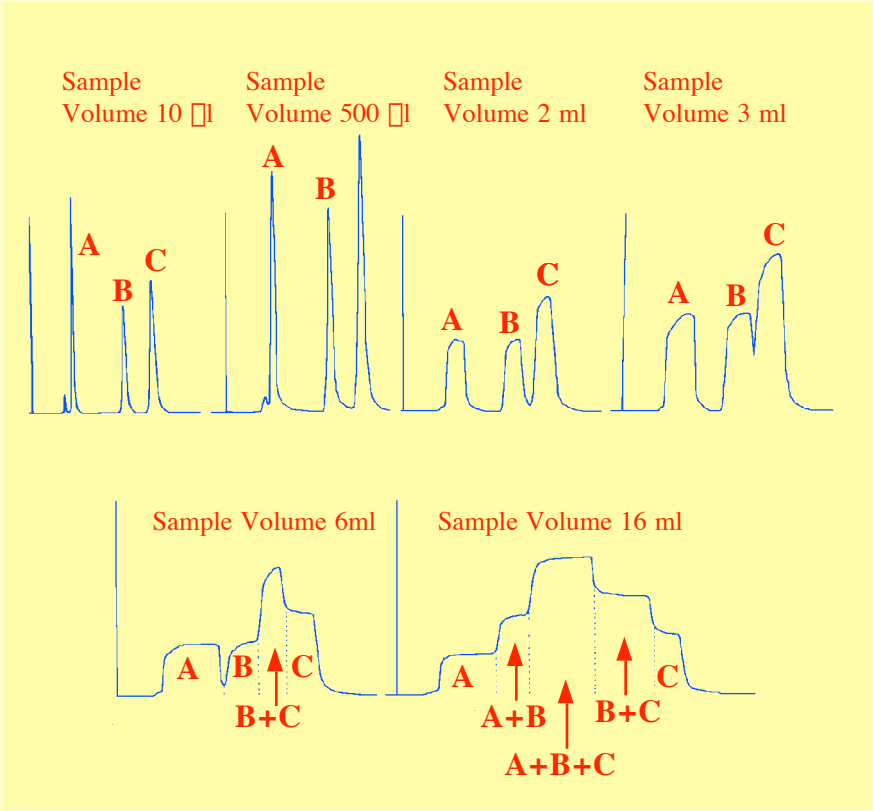


After, J. Chromatogr., Ref. [3]

Figure 4. Retention Distance of Benzene, Naphthalene and Anthracene against Sample Volume

Sample volume overload distorts the normal elution profile derived from normal elution development to that of frontal analysis development. If

elution development is carried out with sample of increasing volume, the distorted elution development concentration profile culminates in frontal analysis.

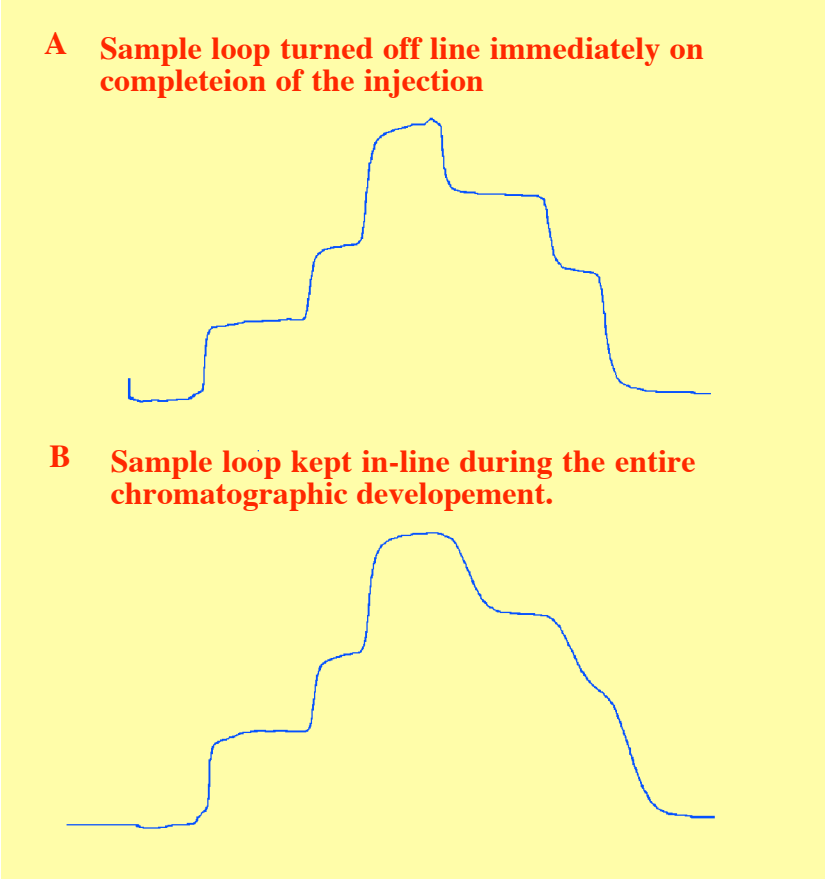


After, J. Chromatogr., Ref. [3]

Figure 5. The Transition from Sample Volume Overload to Frontal Analysis Development.

The transition from elution development to frontal analysis is shown experimentally in figure 5 where progressively larger charges are placed on the column starting with 10 µl and ending with 16 ml. Frontal analysis curves can also demonstrate the need to take certain precautions when employing an external loop valve for injecting the sample. As a result of the parabolic velocity profile of the sample and mobile phase as it is discharged from the sample loop onto the column, dispersion of the sample volume occurs resulting in the concentration profile of the sample entering the column taking the form of a tailing Poisson function as opposed to a rectangular slug. Thus, if the sample loop is left in line with the mobile phase flow subsequent to injection, the peaks will exhibit

serious tailing. This tailing is shown in the lower frontal analysis curves in figure 6.



After, J. Chromatogr., Ref. (3).

Figure 6. Frontal Analysis Curves Demonstrating Dispersion Effects Resulting from Incorrect Sampling Techniques

Examination of figure 6 shows that the descending steps of the curves, which correspond to the tails of the normal elution curves, are very diffuse compared with the ascending steps, which correspond to the fronts of the normal elution curves. If the sample is placed on the column by allowing the mobile phase to flow through the loop for a given time (determined from the flow rate and the volume of sample selected for injection) and the valve then rotated to allow the mobile phase to pass directly to the column, the dispersion effect of the sample tube is virtually eliminated. The improvement resulting from this technique (often called *back-cutting injection*) is shown in the upper curve in figure 6. It is seen that the descending steps of the curve are very similar

to the ascending steps showing that the tube dispersion has been significantly reduced. It should be pointed out that this injection procedure does result in some loss of sample, due to that retained at the walls of the sample tube, but this can be easily recovered and recycled if necessary. This technique is strongly recommended for preparative sampling and should be employed wherever possible.

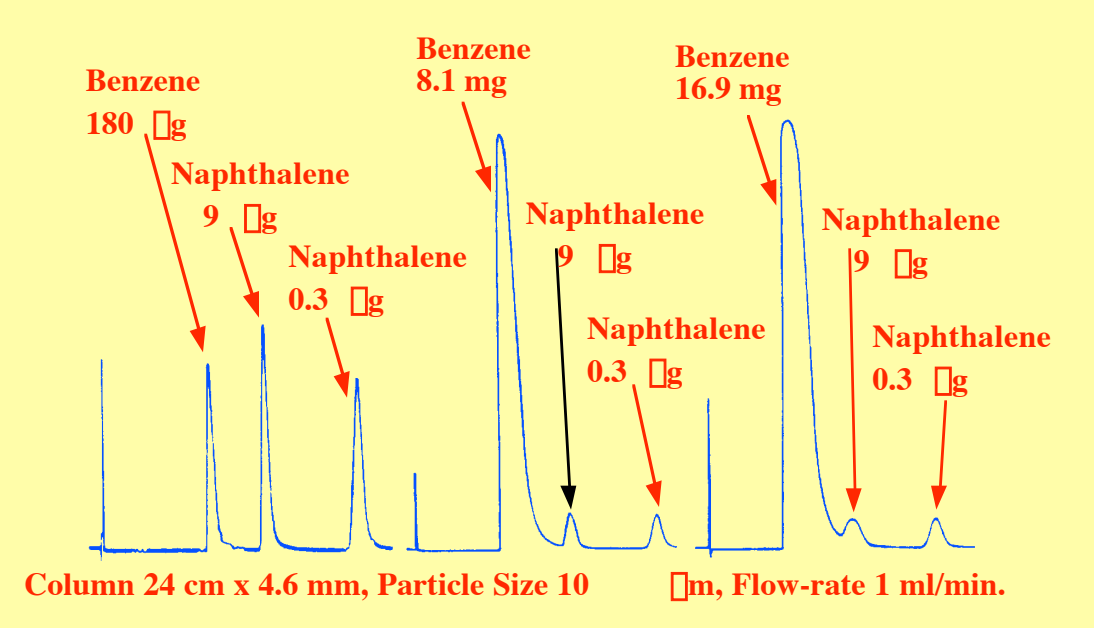
Sample Mass Overload

The effect of excess mass of sample (mass overload) on the chromatographic process can be far more complex than volume overload. The theory of mass overload is, as one might expect, also complicated (5–7) and requires a considerable amount of basic physical chemical data, such as the adsorption isotherms of each solute measured over a wide range of concentration, before it can be applied to a practical problem. Only if the separation problem demands an extremely high through-put, and the process must be as economic as possible, will it be worthwhile to gather the necessary basic data. The problem of mass overload is more conveniently and economically solved by a simple experimental approach.

Depending on the ultimate concentration of solute at the point of injection, a number of effects can take place when a large sample mass is placed on the column. In the first instance, there will be the effect resulting from the limited capacity of the stationary phase. On injection, the sample will spread along the column, carried by the mobile phase, until it contacts sufficient stationary phase surface to allow it to be held on the surface under equilibrium conditions. This will result in a spreading process similar to *sample volume* overload and, if this were the sole contribution to mass overload, could be treated in a similar manner. The peaks would be square topped and similar in shape to those shown in figure 5. However, superimposed on this band dispersion process, is that arising from the deactivation of the adsorbing surface and the change in polarity of the mobile phase due to the presence of the solute. If the charge is substantial, the sample will occupy a significant slice of the column immediately after injection and the adsorbent (stationary phase) will become partially deactivated causing all the solutes in the

mixture to be accelerated through the column with consequent reduced retention times. The increased migration rate is further aggravated by the higher polarity of the mobile phase, which results from the high concentration of the overloaded solute that it contains. This increase in mobile phase polarity causes the solutes to be further accelerated through the column reducing their retention time still more.

These complex effects are best illustrated by experiment. The effect of mass overload was also investigated by Scott and Kucera (3) and the same column was used for mass overload experiments as that employed in the volume overload experiment. In the investigation of mass over load, the sample *volume* was kept constant at 200 µl, and a mixture of benzene, toluene and anthracene was placed on the column, the mass of benzene being increased progressively from 180 µg to 16.9 mg. An example of three of the chromatograms obtained are shown in figure 7.



After, J. Chromatogr., Ref. (3).

Figure 7. An Experimental Example of Mass Overload.

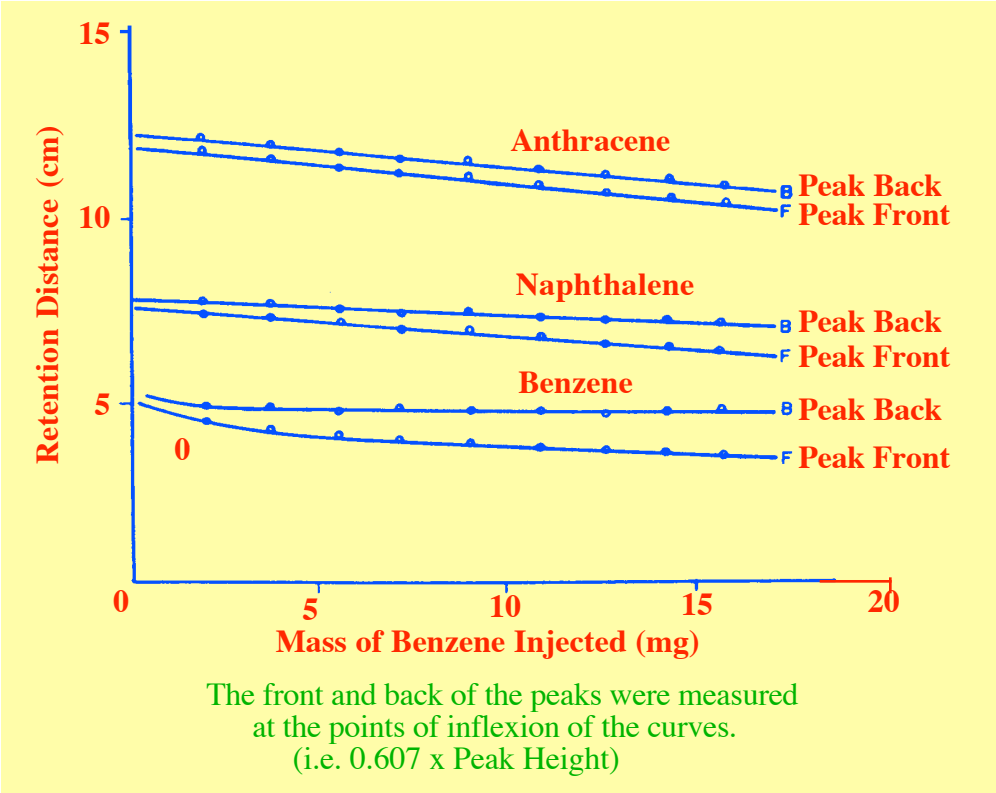
A chromatogram of the reference sample is shown on the left of figure 7 and contained 180 µg of benzene, 9 µg of naphthalene and 0.3 µg of anthracene. The mass of naphthalene and anthracene was kept at 9.0 µg and 0.3 µg respectively for all samples so only the benzene was overloaded. The mass of benzene was increased to 8.1 mg and then to

16.9 mg. Simple visual inspection of the chromatograms indicate that as the mass of solute is increased, the benzene peak has broadened, and become asymmetrical, which will be a direct result of the adsorption isotherm entering the non-linear region. The peak distortion resulting from mass overload is quite clear and it should be noted that, although the retention times of naphthalene and anthracene are both reduced with increased load, the peaks remain relatively symmetrical.

It is also seen that up to 16 mg of benzene could be injected onto the column before the peak merged into that of naphthalene. In fact, by sacrificing a small amount of benzene significantly more benzene (probably at least 30 mg) could have been injected before the major portion of the peak was contaminated with naphthalene. This compromise, which involves sacrificing a small quantity of the selected component to obtain a larger load, is common in preparative chromatography, and arises from the tailing that again results from the formation of a non-linear adsorption isotherm. In samples where the two components are present in the mixture at similar levels and are less well resolved, as in the separation of a pair of enantiomers, this compromise must be taken with caution. As both peaks will be overloaded, and asymmetrical, the tail of one peak will merge with a high concentration at the sharp front of the following peak, and thus significant contamination of the second peak will occur. The results of the overload experiment are better examined quantitatively. Curves relating the retention distance of the front and back of each peak to the sample load are shown in figure 8. The retention distances of the front and back of each peak (measured at the points of injection, 0.6065 x peak height) are shown plotted against sample mass. The change in retention with mass of benzene injected is clearly demonstrated, the maximum effect being for the solute anthracene (the last eluted peak) and the minimum for benzene itself. It is interesting to note that there is little change in the band width of the last eluted peak anthracene.

The different effects of mass overload are now clearly revealed. Firstly, it must be emphasized that the curves in figure 8 represent the movement of the peak extremes and are in no way related to the peak shape. It is

seen that the retention of the rear of the major peak, benzene, hardly changes with sample mass as this represents low concentrations of benzene and thus is eluted in the normal manner.



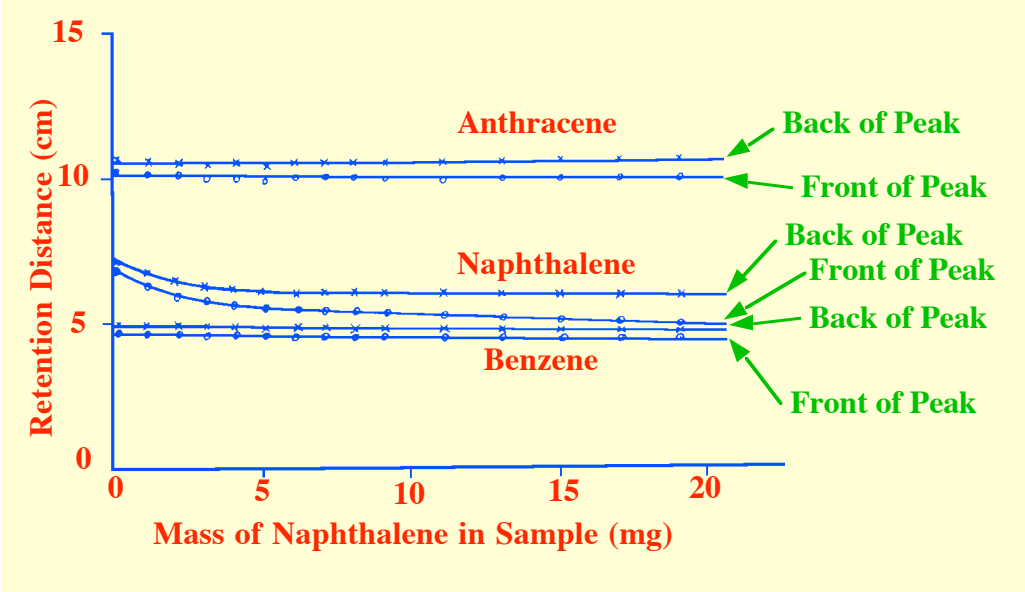
After J. Chromatogr., Ref. (3)

Figure 8. The Effect of Mass Overload of Benzene on the Retention of Benzene, Naphthalene and Anthracene

However, the retention of the peak front is reduced progressively as the sample mass is increased. This results from both the formation of a non-linear adsorption isotherm and the increased elution strength of the mobile phase in contact with the benzene. In fact, as a result of solute–solute interaction the benzene is, in effect, *partially eluting itself.*

The effect of the mass overload of benzene on the other solutes is also clearly demonstrated. The presence of the high concentration of benzene in the mobile phase increases the elution rate of both the naphthalene and the anthracene. Its also seen, however, that the effect of the high concentration of the benzene on the closer eluting peak naphthalene is to produce band dispersion, whereas the anthracene band does not suffer

significant dispersion and the retention of both the front and the rear of the anthracene peak appear to be linearly reduced with sample mass. The chromatograms shown in figure 7 also show that the anthracene peak maintains its symmetry throughout all sample sizes. The impact of the high concentration of benzene on the elution of the other solutes only occurs while the solutes are still in contact with one another at the beginning of the column. Once the separation has started to develop, and the three solutes are no longer in contact, then the naphthalene and anthracene will be eluted in the normal manner. This also explains the significant band dispersion of the naphthalene peak, as, being closer to the benzene peak, experiences the effect of the high concentration of benzene for a longer period and thus, is also effected by the change in character of the stationary phase due to the high concentration of benzene.



After J. Chromatogr., Ref. (3)

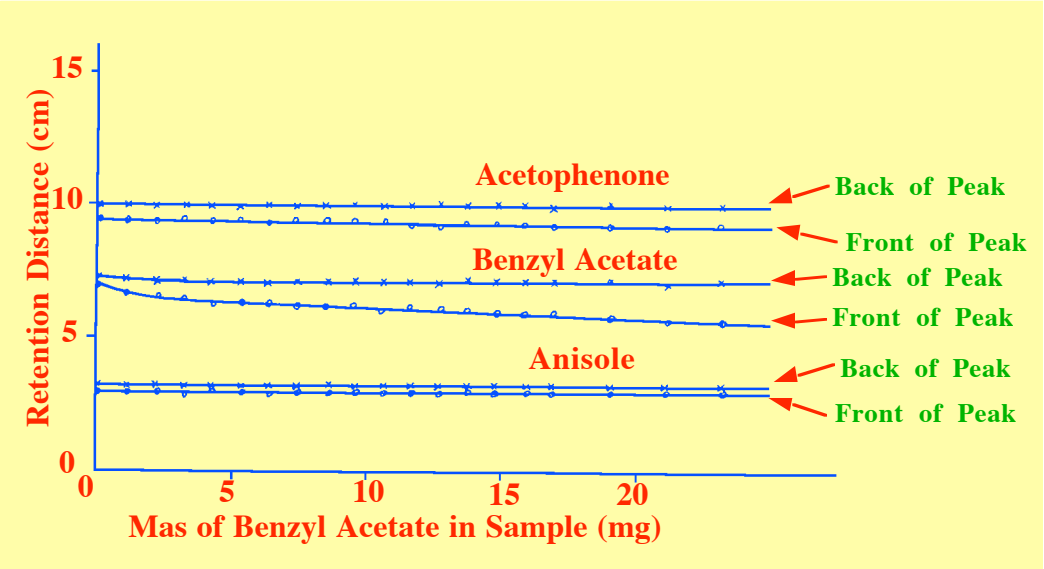
Figure 9. The Effect of Mass Overload of Naphthalene on the Retention of Benzene, Naphthalene and Anthracene

The increased dispersion due to mass overload on closely eluting peaks is of particular importance in the separation of enantiomers and may become the major load limiting factor. It follows, that the phase system that provides the highest separation ratio should be used, irrespective of

any increased (and apparently unacceptable) retention time, as the increase in retention time can often be compensated by the use of multi-sample development (which will be discussed later).

In a similar manner and under identical conditions Scott and Kucera examined the effect of naphthalene over load (from 1.1 to 19.1 mg) on the retention of benzene, naphthalene and anthracene. The results are shown in figure 9.Again the retention time of all the peaks are reduced, but not to the same extent as with benzene overload. This is probably due to the distribution coefficient of naphthalene being much larger than benzene and, although the deactivation of the silica gel will be greater, there will be much less solute in the mobile phase to increase the elution rate. This might imply hat that the greater change in retention from benzene over load arises more from the increased polarity of the mobile phase than from the deactivation of the stationary phase.

A sample of more polar solutes (viz. anisole, benzyl acetate and acetophenone) were examined, employing a 5% v/v of diethyl ether in *n*-heptane as the mobile phase. The reference sample that exhibited no over load contained 19.8 □g of anisole, 44.6 □g of benzyl acetate and 20.5 □g of acetophenone.



After J. Chromatogr., Ref. (3)

Figure 10. The Effect of Mass Overload of Benzyl Acetate on the Retention of Anisole, Benzyl Acetate and Acetophenone

The sample volume was maintained constant at 200 µl and the mass of benzyl acetate increased from 1.1 to 30 mg in a series of steps. A plot of the retention distances of the front and back inflection points of the elution curve of each solute against the mass of benzyl acetate injected is shown in figure 10. It is seen that the curves are very similar to those shown in figure 9. It is seen that there is little change in the retention of anisole or acetophenone as the load of benzyl acetate increases but the band width of acetophenone is significantly increased.

This can be explained on the basis of the relative retention of the different solutes. Anisole is rapidly eluted in front of the overloaded benzyl acetate and, consequently, is only in contact with the portion of the column occupied by the benzyl acetate for a very limited time and thus, is little effected. In contrast the acetophenone is eluted much later than the benzyl acetate and thus, is only effected by the overloading of the stationary phase at the initial stages of development and this causes peak distortion and some retardation. However, as the benzyl acetate moves away from the acetophenone, it is no longer effected, and can be eluted in the normal manner and thus, is not further retarded.

The net effect of mass over load, as opposed to volume over load, is to reduce the retention of the front of all peaks, but the effect is greatest with those solutes that are eluted closed to the over loaded peak. The retention of the back of all the peaks is far less than that of the front. The reduction in retention is by far the greatest for the over loaded peak. It is also clear that in chromatography, column over load is a very effective way of increasing the throughput and by adjusting the selectivity (using temperature, selected stationary phases, or gradient elution) very large sample loads can be tolerated. This approach should always be considered first for moderate loads before contemplating large scale column design.

Preparative Chromatography Apparatus

Very large sample loads will necessitate the use of large scale chromatographic equipment. However, the conventional preparative chromatograph, although certainly more massive, is generally less

complex than the analytical chromatograph. Although gradient elution has been used in preparative chromatography, it should be avoided, if possible, due to the cost of solvents and the complication involved in solvent recovery.

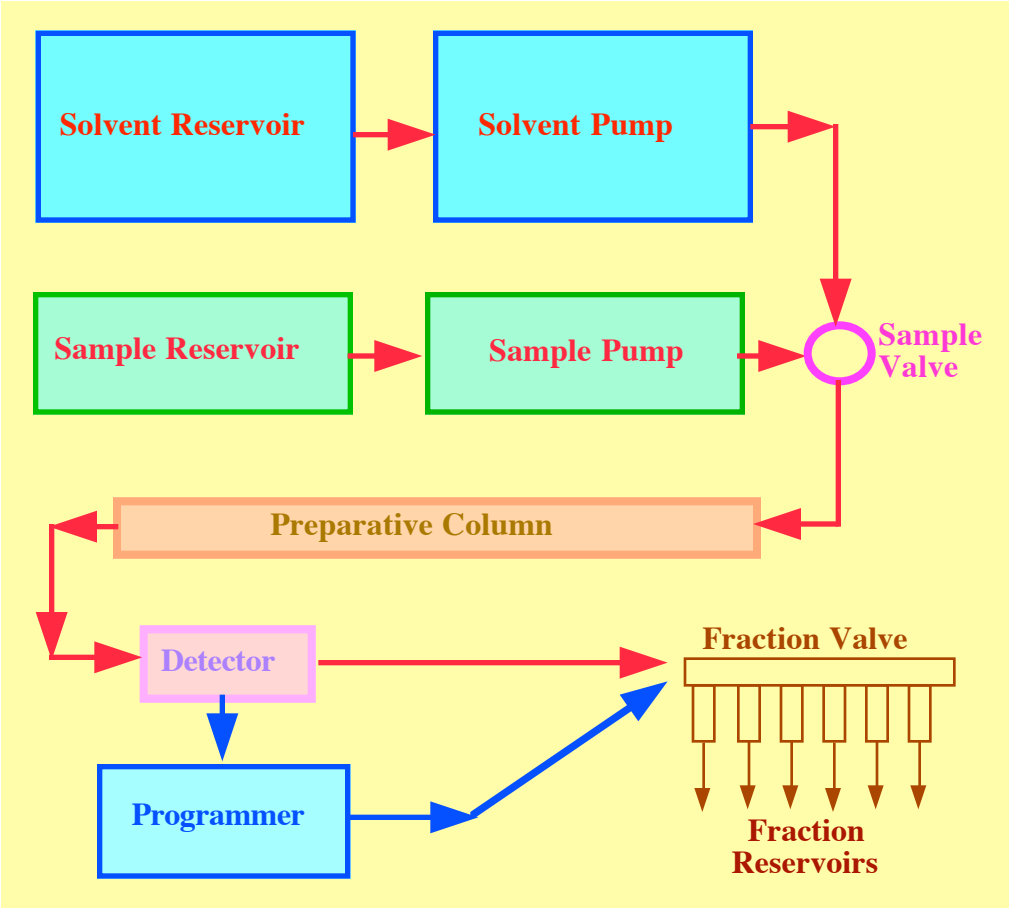


Figure 11 The Basic Preparative Liquid Chromatograph

As a consequence, gradient facilities are not usually fitted to preparative chromatographs. In addition, complex data acquisition and processing systems are not required, although programmable fraction collectors are often necessary which are usually controlled from the detector output. The layout of a preparative chromatograph is depicted in figure 11.

Solvent Reservoirs

The solvent reservoir is usually made of glass or stainless steel and should have an appropriate capacity, normally several gallons. Due to the large volumes of solvent that are often employed, the reservoirs should be closed and fitted with an appropriate 'breathing' device. In general,

most of the parts of a preparative chromatograph are made from stainless steel, however for biologically sensitive, or labile substances, biocompatible materials may be necessary for *certain parts* of the apparatus. To fabricate the bulky preparative components (*e.g.* the column) from titanium that have sufficient strength can become inordinately expensive and plated base metal (*e.g.* using gold or some other suitable biocompatible metal) may be a more economic alternative.

Pumps

The pump must have adequate capacity, and for columns up to 1 in. diameter, pumping rates of at least 150 ml/min. should be available. For larger columns, pumping rates of 500 ml to 1 liter per min. may be necessary. Owing to the limited strength of large diameter columns pressures much above 6000 p.s.i. are rarely required. Any glands involved in the pump should be made from appropriately inert material (*e.g.* 'Teflon' or 'loaded' 'Teflon', polytetrafluoroethylene,). In preparative chromatography, the sample is often placed on the column with a separate pump having its own sample reservoir, usually 1–2 *l* capacity depending on the size of the sample load and the solubility of the materials in the mobile phase. The required volume of sample is often placed on the column by pumping the sample solution at a known rate, for a defined time. In addition to the column and other parts of the apparatus, as the sample pump and reservoir may carry biosensitive materials, it may also need to be constructed of biocompatible materials.

Sample Valves

If the volume of sample is small enough then the sample can be placed on the column with a sample valve. Sample valves, with appropriate loops, can also be used for smaller preparative separations, and sample loop capacities of 20 to 30 ml are practical. Above these volumes, however, a sample pump is recommended. It should also be pointed out that, as already discussed, there can be considerable dispersion in open tubes, and the sample loop should not be left in-line during development. The valve should be returned to the 'flush' position immediately after injection, so that the 'tail' of the sample left in the loop does not cause dispersion and tailing on the column. This procedure may entail sample recovery from the residue contained in sample valve.

Preparative Columns

Preparative columns (GC or LC) are usually made of glass or stainless steel the latter being used for high pressure systems. Preparative columns must be designed to accommodate the inlet pressure necessary to obtain the required flow rate through the packed bed which is determined by the size of the particles selected for the packing.

The larger the column diameter, the stronger must be the column and the thicker the walls. Large column operating at high pressures with relatively small particles can become extremely bulky and heavy. In addition, the construction of wide columns (3 in. O.D. and greater), irrespective of the packing, can be extremely expensive to both construct and to pack and it is essential to take cost into all design considerations.

Columns having diameters greater than 0.5 in. need to have the frit supported on a suitable grid, as the frit material has limited strength and will fracture under pressure. The porosity of the frit will be determined by the particle size of the packing. In order to minimize the pressure drop across the frit at high flow rates, the frit porosity should not be made unnecessarily small. Radial dispersion of the ample can be extremely slow (see book 9 of this series) and thus a device must be used to distribute the sample across the column so the full loading capacity of the column can be realized.

This is achieved by the use of a sample distribution plate a diagram of which is included in figure 12. It consists of a disc in which is cut a series of radial slots and can be used for both GC and LC. The sample is placed on the top of the disc where it is quickly distributed across the plate surface and then onto the column through the slots. In this way the sample is injected across the top surface of the packing.

Preparative Detectors

Preparative chromatography detectors can have very limited specifications, compared with their analytical counterparts. They need not be particularly sensitive (in fact too great a sensitivity is a distinct

disadvantage) as the sample size and the eluent solute concentrations are very large.

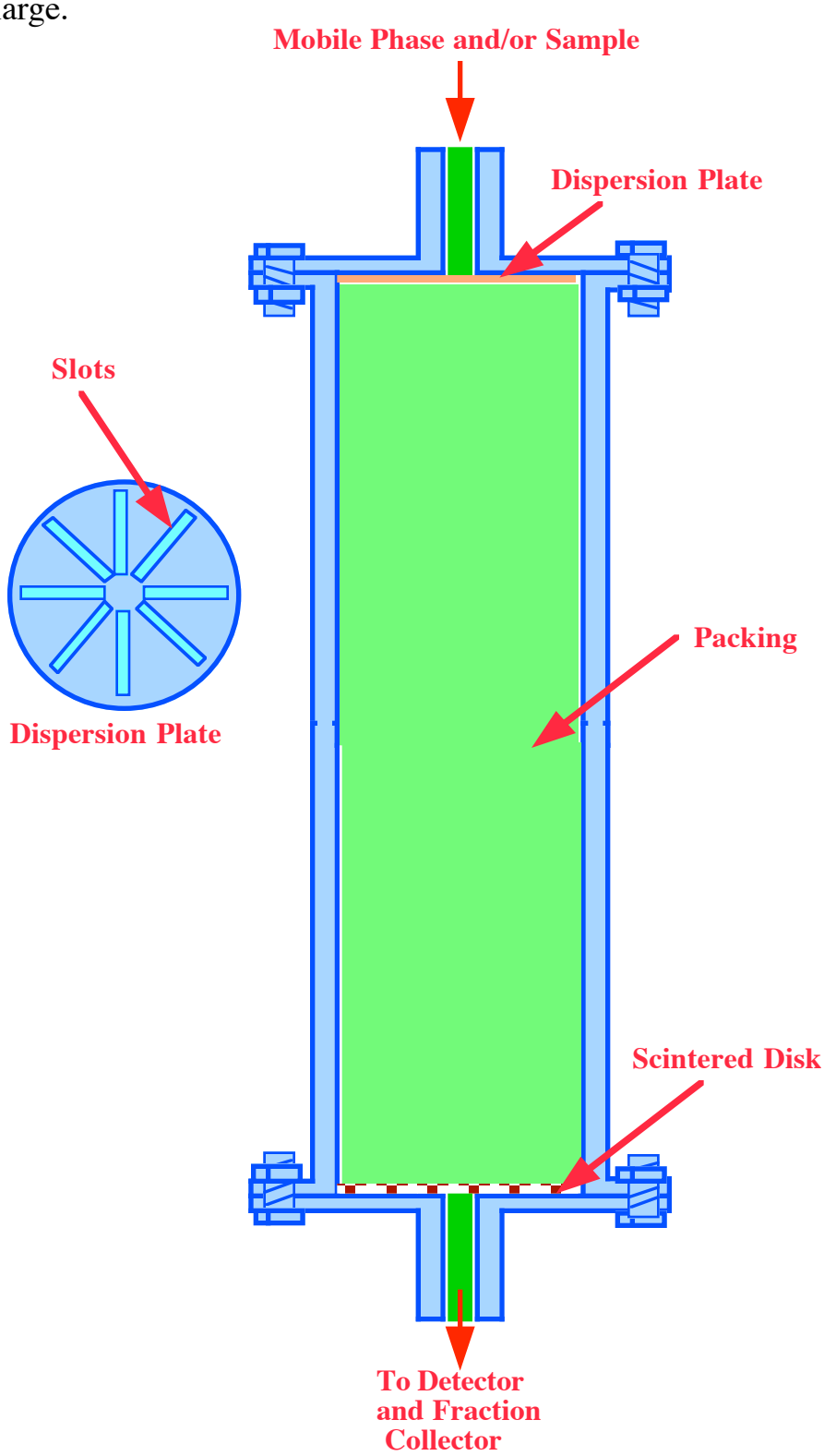


Figure 12. A Preparative Column

Preparative chromatography detectors can have large sensor volumes and, as the detector is required only to monitor the separation, they need not have a linear response. They do need to tolerate high flow rates and thus, must have low flow impedance. Analytical detectors can be used for preparative purposes but a portion is usually split from the column eluent, diluted with more mobile phase and then passed through the detector. In practice this becomes a rather clumsy procedure.

The most commonly used detector in preparative GC is the thermal conductivity detector (hot wire detector). Even this detector, however, is often too sensitive and has too high a flow impedance. Under such circumstances, the procedure mentioned above must be employed. The eluent from the preparative column is split and a small portion diverted through the detector (sometimes with further dilution with carrier gas to reduce sensitivity).

In LC, the refractive index detector is probably the most useful of the analytical detectors for preparative work, but even at its lowest sensitivity setting, it may still be too sensitive. The multi-wavelength UV detector can sometimes be used providing its conduits can tolerate the necessary high flow rates. A very short path length cell must be installed, or a variable path length cell can be used. Another option is to set the operating wavelength at that which the solutes have very small extinction coefficients so that the detector has a very weak response. If possible, a detector specifically designed for preparative work should be used, but there are a very limited number of these available.

An example of a very simple fixed-wavelength detector, suitable for use in preparative chromatography, is shown in figure 13. This detector was invented by Miller and Strusz (8) and was originally manufactured by GOW-MAC Instruments. In the device shown in figure 13, the column eluent passes through a delivery tube and over a supporting plate that is either very thin, or made of fused quartz, so that adequate UV light can penetrate and reach the photo cell situated on the other side of the plate. The liquid flows over the plate and the effective path length is the film thickness peculiar to the flowing solvent layer. The UV lamp is situated on one side of the plate and the photo cell on the other side, each facing

normal to the plate surface. A reference photo cell (not shown) is placed close to the lamp to compensate for changes in light intensity that may arise from variations in lamp emission.

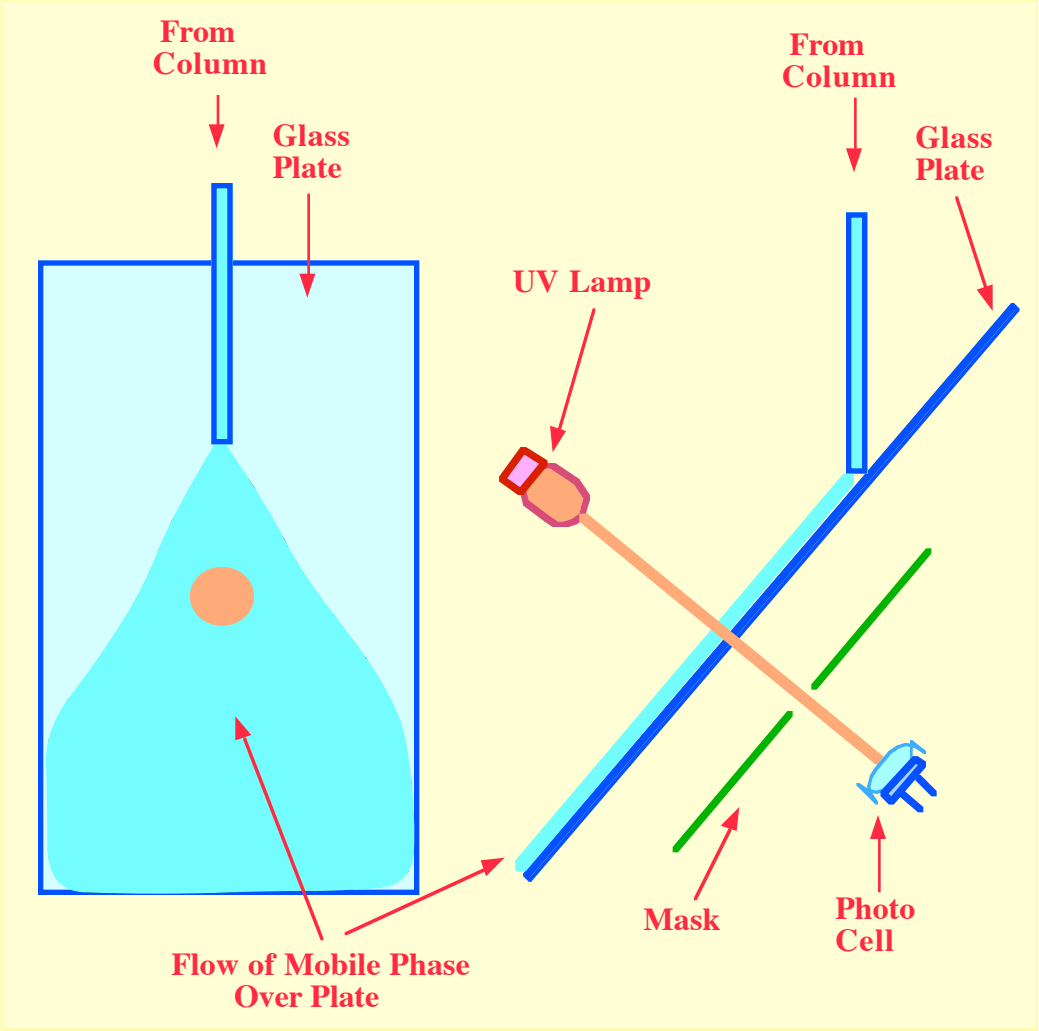


Figure 13 Fixed Wavelength UV Adsorption Detector for Preparative Work

Due to the very short path length (the thickness of the film of column eluent) the detector has the required low sensitivity and low flow impedance. The detector can operate very satisfactorily at concentrations as great as 10^{-2} g/ml (1% w/w), which is ideal for preparative chromatography. Another advantage of the device for preparative work, is its very low flow impedance and thus can easily cope with the high flow rates used in preparative LC. The film thickness does depend,

among other things, on the column flow rate and thus fairly precise flow control is necessary for the satisfactory performance of this detector.

Fraction Collectors

Fraction collection in preparative chromatography is usually achieved using a multi-port valve and a number of collection vessels. The selector valve should be programmable on the basis of time, or be actuated by the detector output signal (preset at the appropriate signal level or signal derivative) or, preferably, both. The valve should have at least six ports, or preferably ten. If the system is to be used solely to separate a binary (*e.g.*, a mixture enantiomer pairs) then a six port valve would usually be adequate.

Depending on whether the mobile phase is a gas (as in GC) or a liquid (as in LC) the solute is extracted from the eluent by condensation or adsorption. In the case of LC the sample can be collected as a solution in the mobile phase and recovered by evaporation. In GC, depending on the partial pressure of the eluted solute, the material can be collected by cooling with a mixture of solid CO_2 and acetone or, in the extreme, with liquid nitrogen. In cases where the material collected is very valuable, argon can be used as the carrier gas and both the solute and the argon mobile phase condensed in a liquid nitrogen cooled trap. The argon is then carefully allowed to slowly evaporate leaving the collected solute as a residue. To simplify the recovery procedure in LC and reduce the volume of solvent that must be evaporated during product recovery, alternate ports of the multi-port selection valve should be connected to waste.

Product recovery when using normal phase solvents is best carried out by bulking the fractions and removing the solvent in a rotary evaporator under reduced pressure. For reverse phase solvents that have a high water content, recovery can be best achieved by passing the fraction through a reverse phase, C18, column of high capacity.

The solute and solvent is adsorbed, and the solute and solvent content of the fraction can be recovered by displacement with another solvent, and the solute, now concentrated is recovered by evaporation.

Solvent Hazard

Unless solvent recycling can be employed, which is not always possible, the operation of large diameter columns inevitably involves the use of large quantities of solvent. It follows, that there may be a possibility of both fire and toxicity hazards. As a consequence, the solvent should be selected with care and if possible the entire chromatograph, including the solvent supply, electrically grounded, and the apparatus including fraction reservoirs should be located in a walk-in fume hood.

Packing Preparative Columns

A number of packing techniques are available to the chromatographer that can be used to prepare preparative columns. The best and most appropriate method for any particular column system will depend partly on the particle size of the packing, partly the scale of the separation and partly on the nature of the material to be separated. Contrary to popular belief, there is no magic involved in packing a column, but some experimental skill is required together with a considerable amount of patience, in addition some experience also helps.

GC Columns

Small GC columns, up to 2 or 3 ft long and up to 0.5 in. in diameter can be hand packed using techniques similar to those used in the preparation of analytical columns. The system used is shown on the right hand side of figure 14. The column is fitted with a *pre-column* so that the column proper can be packed 3 or 4 inches longer than is actually required. The column is packed in a vertical position by adding small quantities of packing (about 2 ml) at a time and tapping the sides of the column until the bed settles to a minimum volume. This procedure is continued until the system is completely packed.

The pre-column is then carefully removed from the actual column and the top flange connected. The pre-column ensures that the top of the packing will be packed to a similar density to the bulk and will not be 'loose' and more porous and permeable. For larger columns the apparatus on the left of figure 14 can be used. This procedure was developed by Filipi (9) for small bore GC columns but works equally

well for large preparative columns. Sufficient packing is placed in the top reservoir to pack about 110% of the column volume, so that after packing the reservoir still contains a significant amount of packing.

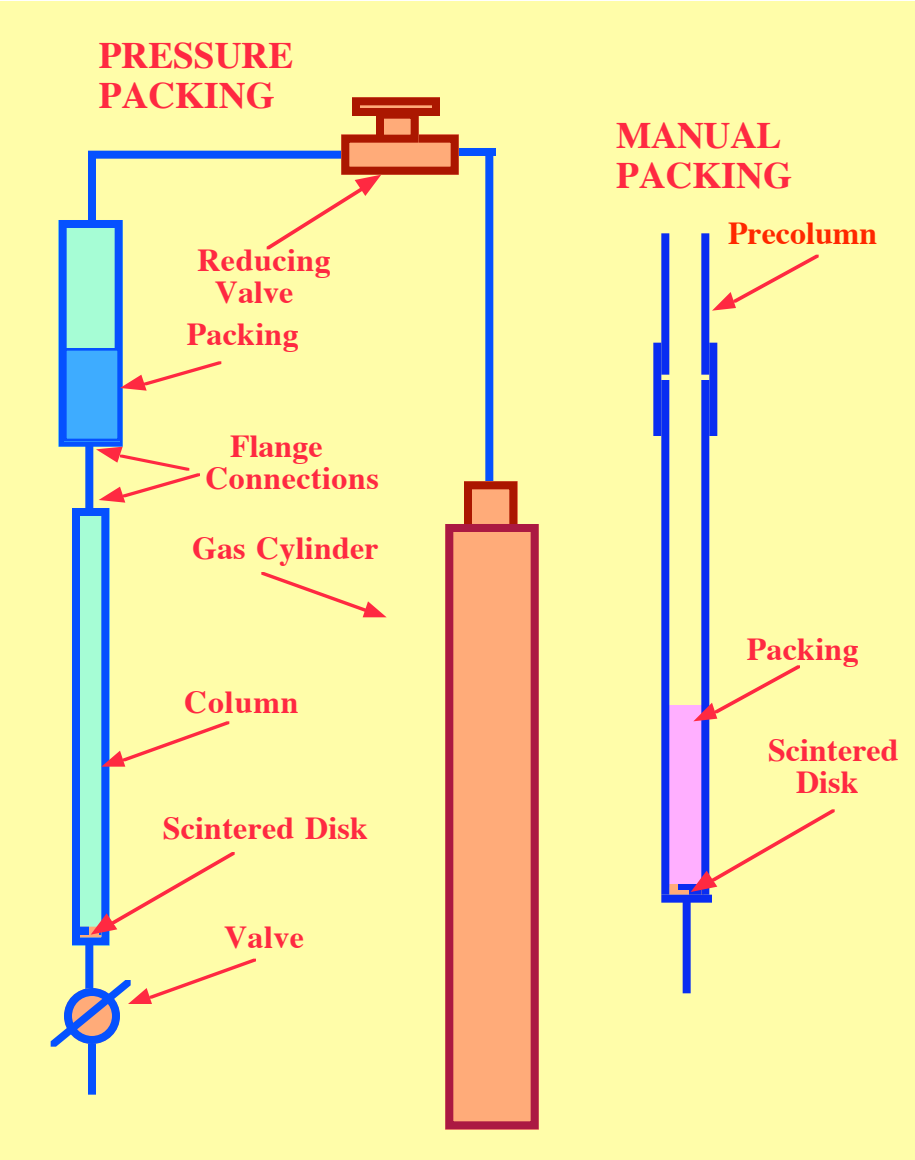


Figure 14. Apparatus for Packing Preparative GC Columns

The reservoir is then connected to a reducing valve and gas cylinder (tank). The valve at the column exit is closed during this period. The pressure in the system is very slowly brought up to a pressure of about 50 p.s.i. (the optimum pressure will depend to some extent on the particle size of the packing and the column length and may need to be determined by experiment. During the initial pressure adjustment some

of the packing passes into the column and forms a lightly packed bed at the bottom of the column. The exit valve is hen rapidly opened and the sudden flow of gas packs and compacts the bed at the same time. After packing, the reservoir is carefully removed so as not to loosen the top of the packing and connected to the sampling system.

LC Columns

If particle sizes in excess of 20 μm are used, then the column can often be dry packed, with appropriate tapping, or, even better, with longitudinal and radial sonic vibration. The variance per unit length obtainable from a preparative LC column should be less than 2 particle diameters (determined using analytical scale samples). It is worth remembering that (as already discussed) when designing preparative columns, it is better to obtain the necessary efficiency using a longer column packed with larger particles, than the converse. The long column will permit much larger charges and, if pertinent for the sample concerned, will also allow multiple sample development techniques. In addition, the larger particles will provide greater column permeability, and thus lower pressures can be used. Lower pressures will, in turn, allow lighter and less expensive materials to be used in the construction of the preparative system.

LC columns employing particle diameters less than 20 μm are almost universally 'slurry' packed. The apparatus used is similar to that used for GC columns, but a pump and reservoir is used in place of a gas tank. A diagram of the apparatus is shown in figure 15. When slurry packing preparative LC columns, care must be taken not to operate at pressures in excess of the bursting strength of the tube used for the column. As the column diameter increases, the maximum permissible pressure rapidly falls unless extremely thick walled tubing is employed. The safe maximum pressure for any tube can usually be obtained from the tube or column supplier. A slurry is made of the packing (110% of that needed to fill the column) and placed in the packing reservoir. The reservoir is rapidly connected to the pump (which must have both an adequate delivery rate and an adequate operating pressure) the delivery

rate will depend on the column diameter and the applied pressure on the wall thickness and the column length).

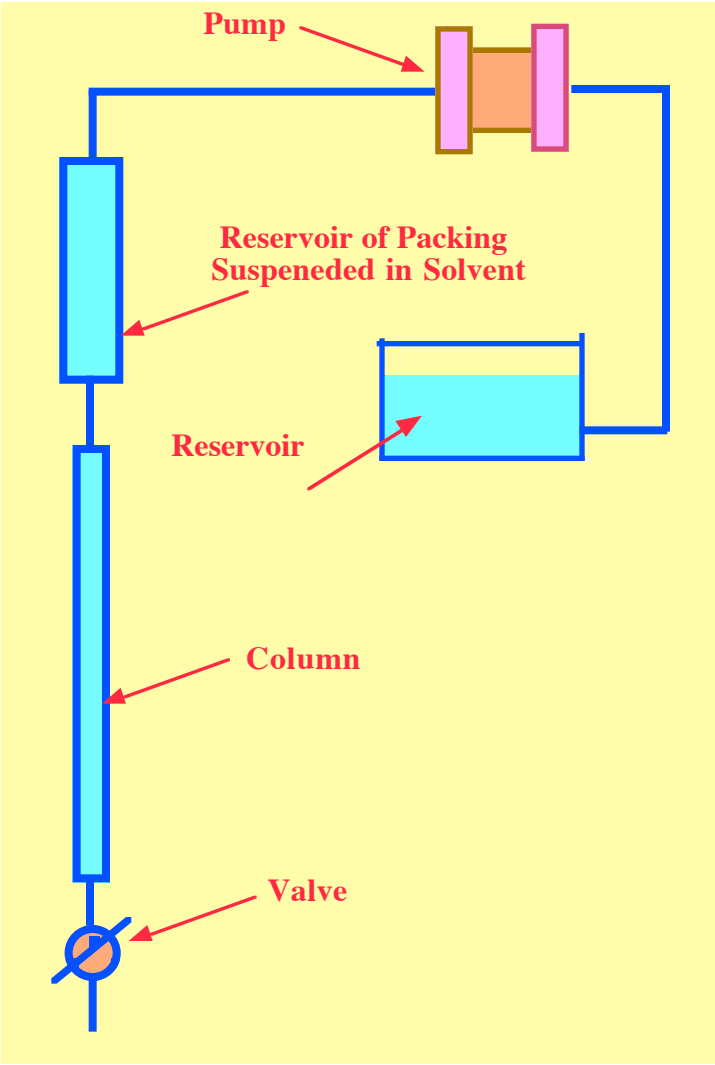


Figure 15. Apparatus for Packing Preparative LC Columns

The pump is started and the column exit valve opened and the flow continued until it has been reduced to a constant value. The flow is then arrested and some packing will remain in the reservoir which ensures that the top of the column is tightly packed. The column is disconnected, the packing secured with a suitable frit, and then connected to the chromatograph.

The major difference in equipment used for larger scale chromatography lies in the technology required to pack and maintain high-performance

LC columns of large diameter. Another difference is that industrial-scale equipment is often dedicated to just one specific separation.

Once the diameter of an HPLC column approaches 5 cm, additional difficulties arise in its preparation and operation. It is possible to pack a column 5 cm in diameter by conventional high-pressure slurry packing techniques but this may not be easy to do, particularly in a laboratory environment, and it becomes even more difficult and expensive for columns of wider diameter. There are other problems that need addressing, once packed, the practical lifetime of a column is also uncertain. The changes in performance of a preparative HPLC column that occurs with time depends upon the stability of the packed bed. Frequently, the bed settles after operation for even a short time and the top of the column needs to be repacked. Sometimes channels are formed in the bed, in which case the entire column has to be repacked. The rate of settling again depends upon the diameter of the column. This bed instability arises because there is a significant change in wall support as the column diameter increases. In analytical columns the walls are relatively close to the center of the column and 'bridges' of packing particles can be formed across the bed, as shown in Figure 16. These bridges allow the longitudinal forces acting on the packing within the column to be dissipated to the walls. When a column is packed, it is never in its optimal configuration and there are always areas where the packing is not as tightly packed as could be desired. The presence of the bridges protects the bed from the full pressure of the system, stabilizing the more poorly packed areas. When wider columns are used, the supporting bridges can no longer form, and the metastable packing areas are no longer conserved by bridges and are free to rearrange. This is a function of both column and particle diameters and also the frictional forces between particles and the wall. The lack of wall support makes the whole bed 'active' in that it is not only free to rearrange but also particles (usually the very small 'fines' in the packing) can move through the bed. The rearrangement of the more poorly packed areas results in the formation of voids or channels in the packed bed, while the migration of fines results in sudden increases in operating pressure with time which are sometimes a feature of large columns. The problem of bed stability in

large diameter columns has been addressed by use of technologies which support the packed bed and allow changes in bed volume to be accommodated without effecting column efficiency.

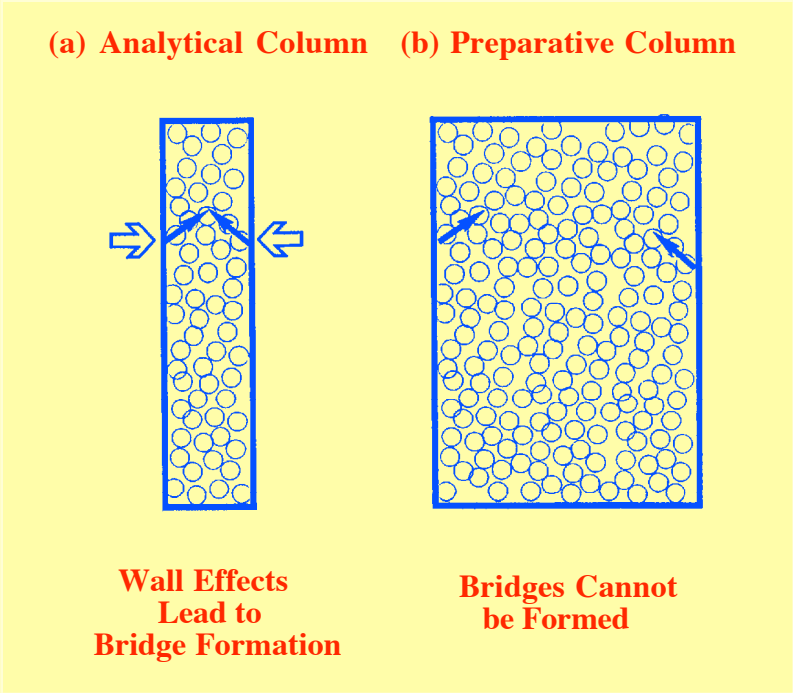


Figure 16 Reduction in Bed Stabilization by Wall Effects in Preparative Columns

For production columns, the compensation for the variation of bed volume must be automatic, since it is clearly poor practice to wait for a problem to arise before taking avoiding action. Two techniques are in wide use, both relying upon some form of compression system to preserve the bed lifetime the radial compression column and the longitudinal compression column. The principles of both techniques are schematically shown in figures 17 and 18.

Radial Compression

In the radial compression column, shown in figure 17, the column walls are flexible, which is achieved by packing the bed into a tubular polymer cartridge (terminated by an appropriate stainless steel frit). The cartridge is contained inside a supporting stainless steel cylindrical column. The column is filled with a very dense slurry of the packing material and another frit fitted to the top of the bed. The bed is then compressed

radially by applying pressure to the outside walls of the polymer cartridge. The applied pressure may be either pneumatic or hydraulic.

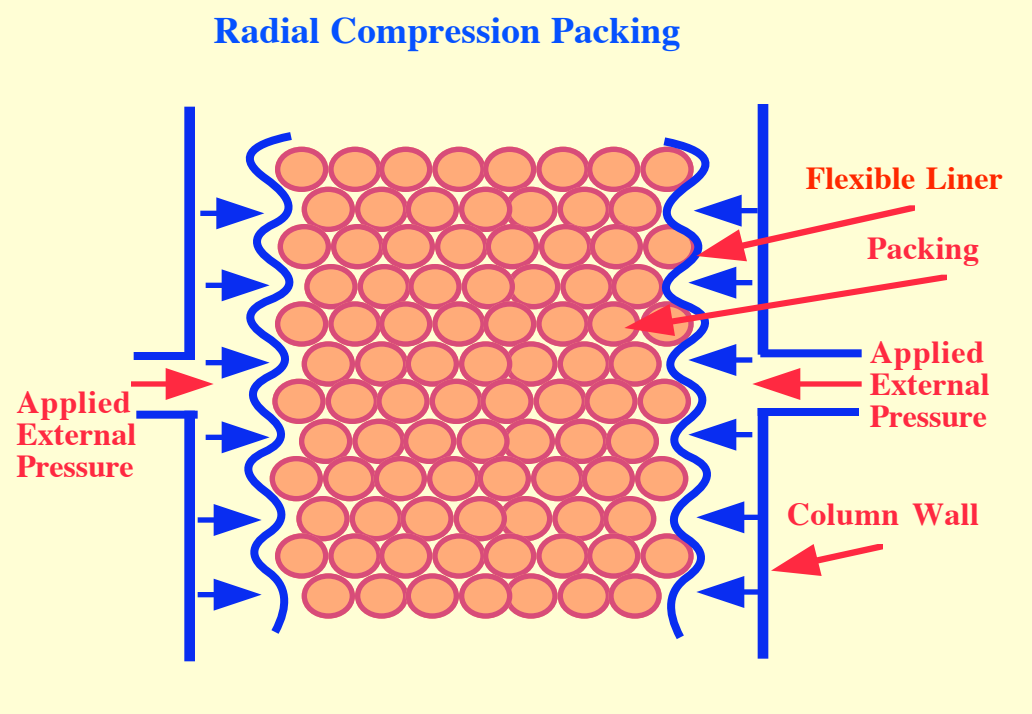


Figure 17 Radial Compression for Preparative Column Bed Stabilization

This technique will automatically take up bed volume changes (provided they do not exceed the limits of flexibility of the cartridge) and can automatically compensate for the bed reorganization during operation. The radial compression technique is generally applied in columns of up to 20 cm (8 in.) in diameter.

Axial Compression

The alternative technique for bed stabilization by compression is to use an axial rather than a radial force. Because any density gradients caused by the compression are longitudinal rather than radial they will not affect the flow profile along the bed. Thus, the bands remain undistorted and very high efficiencies can be obtained with this technology. In addition, there is no restriction on column diameter, and HPLC columns of this type with diameters as large as 80 cm have been made. A diagram of the axial compression packing system is shown in figure 18. The apparatus

consists of a column (capped at the top with a stainless steel frit) having a precisely controlled internal diameter and finish.

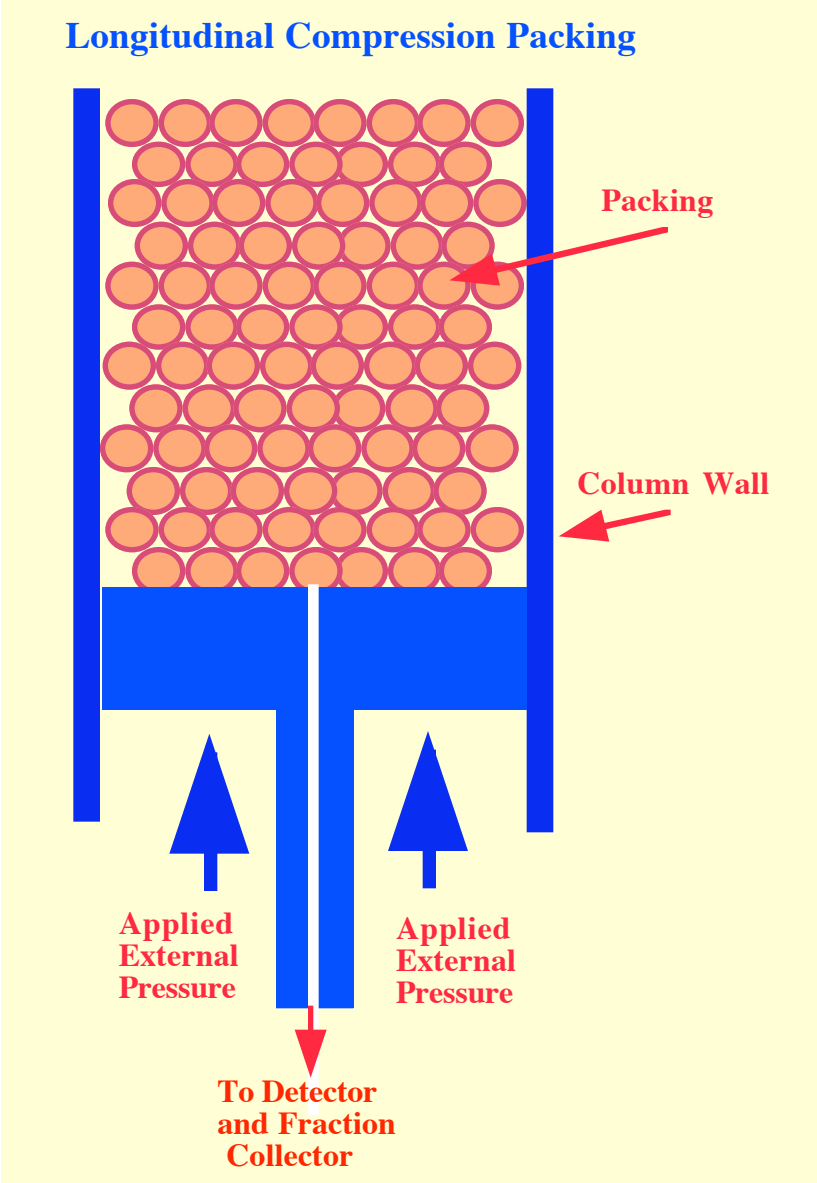


Figure 18 Longitudinal Compression for Preparative Column Bed Stabilization

The column contains a close fitting piston. The piston, which is mounted on a constant-pressure hydraulic jack, can be moved throughout the entire length of the column. It contains a porous frit at the top and a channel for passage for the mobile phase through its center. The channel is connected to the detector and thence to the fraction collector. To pack the column, the piston is withdrawn to bottom of the column and the

column filled with the measured mass of packing. The amount of packing used will determine the effective length of the column. The hydraulic jack is activated and the packing compressed to the maximum pressure which is determined by the mechanical strength of the packing. If the packing is crushed by the use of too great a pressure, many fines will be produced, which cause the permeability to be extremely low and seriously reduce the column efficiency. The pressure to the column is maintained continuously and during each separation. In addition to stabilizing the bed when packed, the system allows the rapid packing and unpacking of media which can range from gel packings such as cross–linked agaroses to 8 μm silica.

At the extreme, these techniques can allow the scale of preparative chromatography to be much increased and can permit intrinsically expensive materials (such as antibiotics and other biotechnology products) to be processed in kilogram quantities and more. There are, however, other methods of increasing the scale of chromatographic separations

Recycling Development

A method for improving the resolution of a pair of closely eluting solutes (*e.g., a* pair of enantiomers), on a preparative column of limited length, is to employ the technique of *recycling*. A diagram of a recycling system is shown in figure 19. The basic procedure is as follows. The two valves are set so that solvent from the reservoir is pumped through the injection valve to the column and the column outlet valve is set to the fraction collector. After the sample has been placed on the column, the column outlet valve is set to pass the column eluent back to the pump and the solvent selection valve set to the column eluent. Thus the mobile phase is continuously circulated through the column, through the detector, back to the pump and then back to the column. As a result the column is used over and over again many times, and each time the sample passes through the column, the resolution is improved. Unfortunately, the resolution is not necessarily proportional to the number of cycles, as significant peak dispersion can occur each time it passes through the pump. Nevertheless, there is a substantial net gain in resolution on each

cycle. This procedure can be very time consuming if long retention times are involved, but has the great advantage of solvent economy.

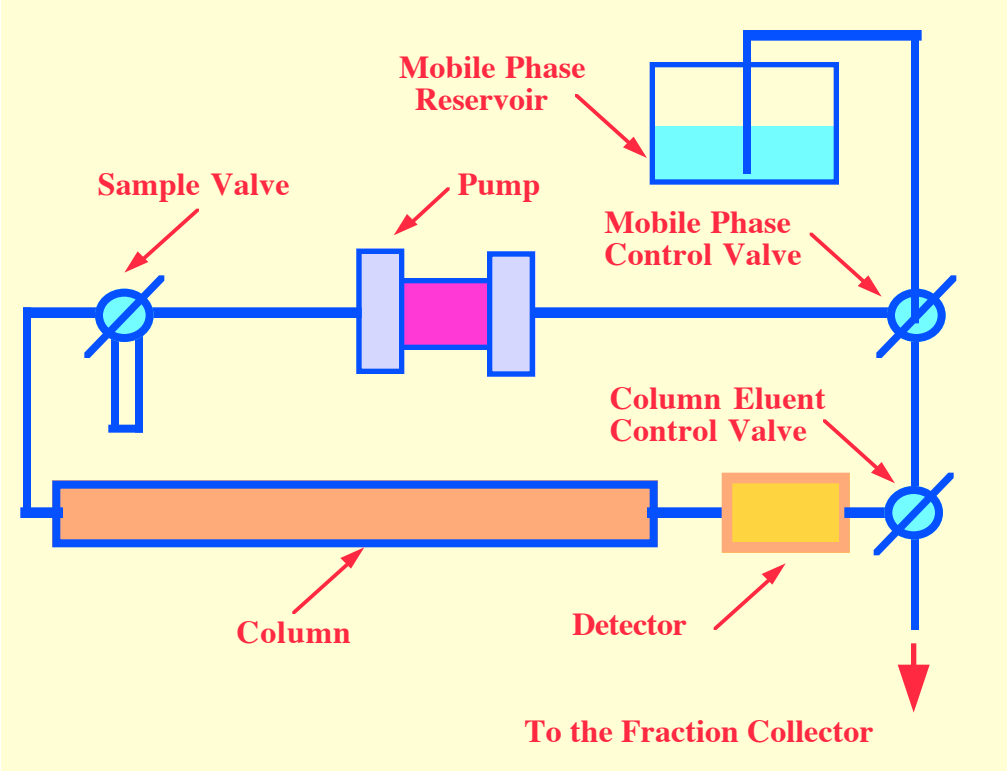
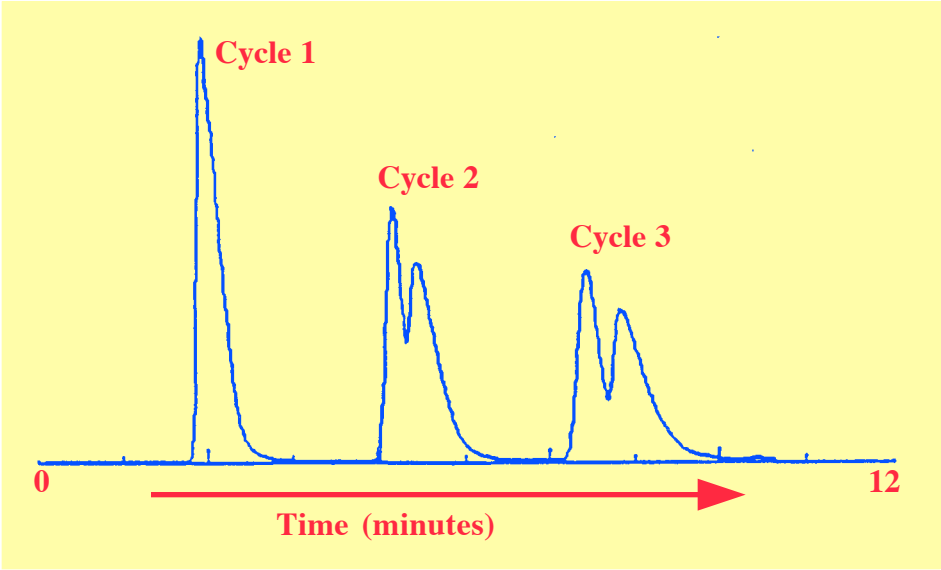


Figure 19. The Apparatus for Chromatographic Recycling

The recycling procedure, in effect, artificially increases the column length and actually trades in *time* for *solvent economy*. An example of the recycling process is demonstrated in the separation of the Warfarin enantiomers shown in figure 20. The separation was carried out on a CYCLOBOND 1 2000 column, 25 cm long, 1 in, I.D., using a mobile phase consisting of methanol/acetic acid/triethylamine : 100/0.3/0.2 v/v/v, at a flow rate of 12 ml/min. The separation of a simple binary mixture of chiral isomers is an excellent example of the type of separation for which the recycling technique is particularly useful.

The chromatogram shows the results obtained from three complete elution cycles. In the first cycle, although separation has begun, there is little or no visible resolution. It should also be noted that the peak is distinctly asymmetrical. On the second cycle, the two enantiomers are beginning to separate and the asymmetry is at least as bad if not worse. In the third cycle, the separation is improved still further, and is sufficient

to allow the collection of significant quantities of the individual isomers at a high purity despite the asymmetry. It is also seen that the process is fairly rapid as three cycles are completed in less than 12 minutes.



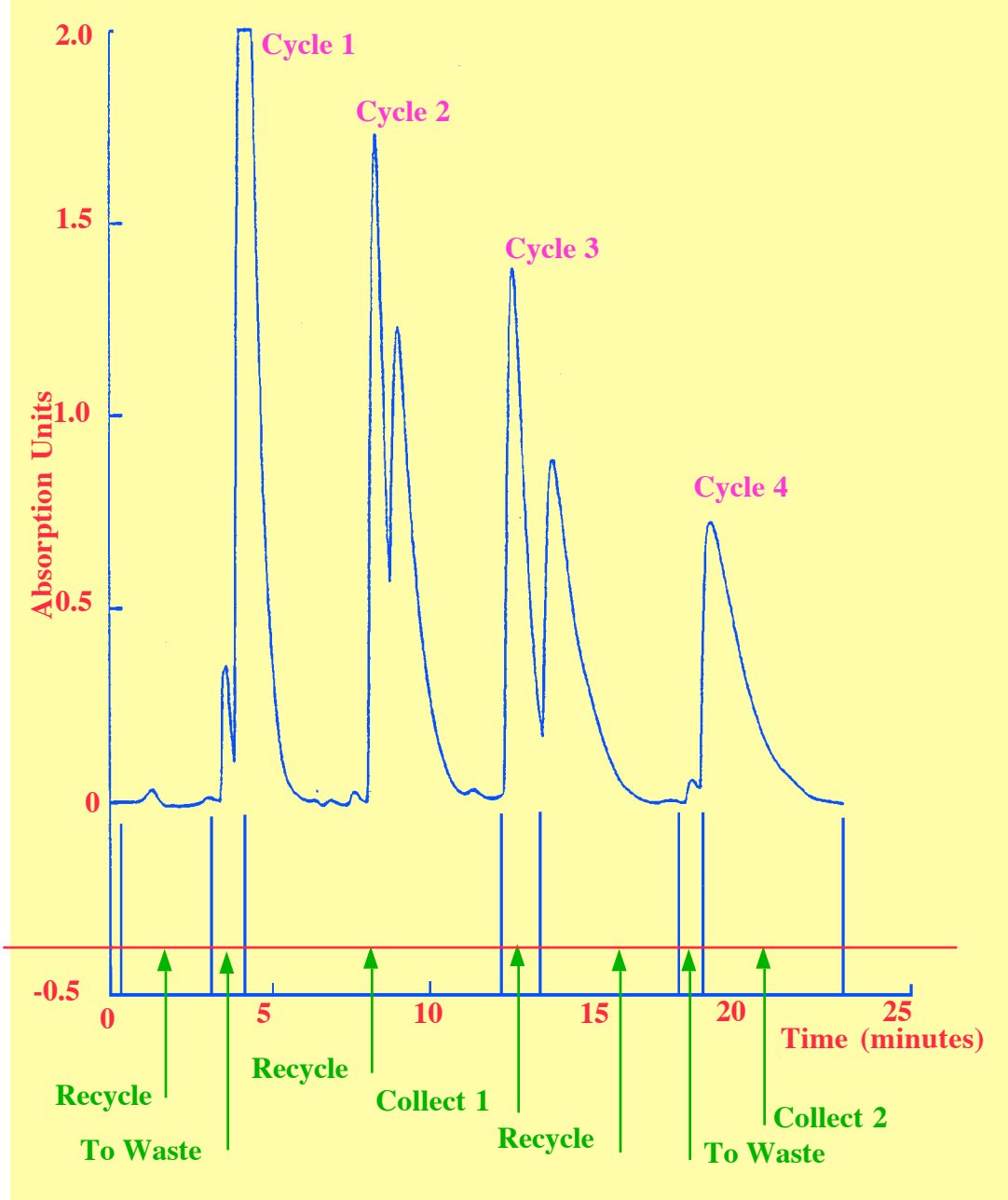
Courtesy of ASTEC Inc.

Figure 20 The Separation of the Warfarin Enantiomers by Recycling

An additional example of the technique of recycling, which includes another collection procedure called *peak shaving*, is shown in figure 21 which depicts the preparative separation of the enantiomers of 5-methyl-5-phenylhydantoin.

The recycling and peak shaving procedure is included in figure 21. It is seen in figure 21 that after the first cycle, there is very little resolution of the enantiomers, but an impurity is separated on the front of the composite peak. This peak is diverted to waste a procedure that is termed *peak shaving* (from the main peak). During the second cycle, the separation of the enantiomers is beginning, although the isomers are insufficiently resolved for peak collection to be initiated. During the third cycle, the first major peak is 'shaved' from the composite peak. The column was 30 cm long, 2 in. I.D., packed with 10 □m particles carrying Vancomycin as the chiral stationary phase. The mobile phase was ethanol

and the flow rate 100 ml/minute. The sample load was 400 mg dissolved in 5 ml of ethanol.



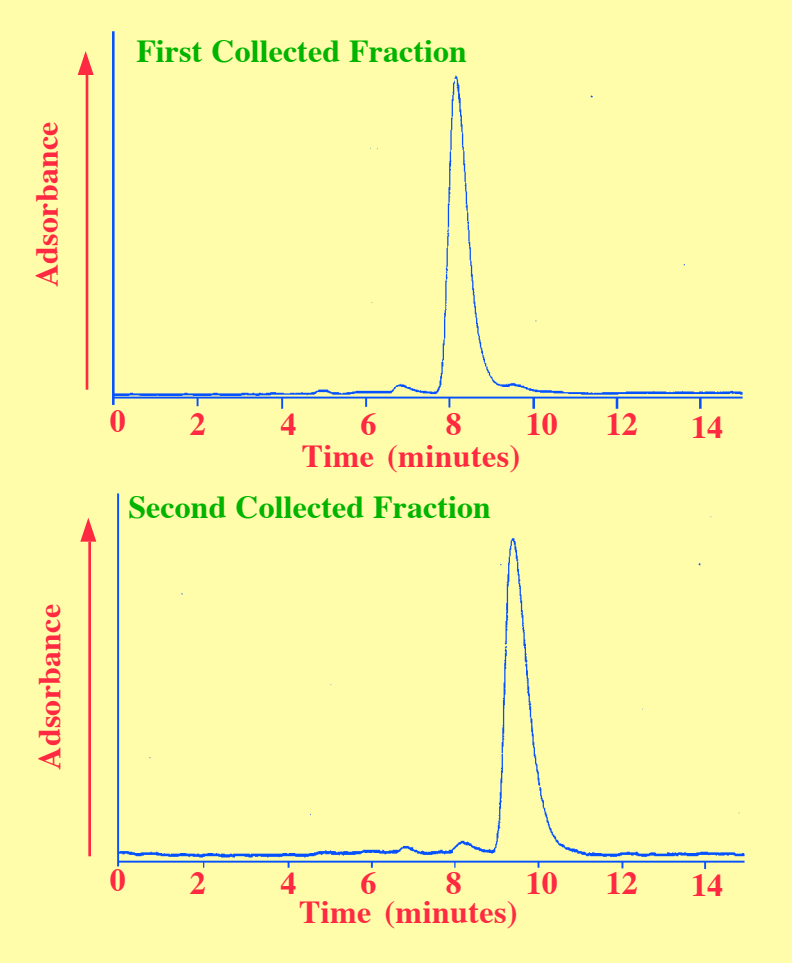
Courtesy of ASTEC Inc.

Figure 21. The Preparative Separation of the Enantiomers of 3-Methyl-5-Phenylhdantoin

It is important to note that as *the overloaded peak is asymmetrical and tails*, the first peak will be collected *virtually pure*. The second peak will

remain contaminated with a small amount of the first peak. After the fourth cycle is complete, the trace of the first isomer is shaved from the major peak and passed to waste. The remainder of the peak is then collected with little or no contamination. The separation is fairly rapid, the four cycles are completed in less than 25 min. and, thus, the output of the system would be nearly 1200 mg per hour. The recovery of each enantiomer was 98% and had an enantiomeric excess of 98%.

In addition, this procedure can be fully automated, providing the recycling and collection program is controlled by the detector signal.



Courtesy of ASTEC Inc.

Figure 22. Chromatograms of the 5-Methyl-5-Phenyl-Hydantoin Enantiomer Fractions

Alternatively the separation can be programmed on the basis of time but the reference points must be re-adjusted if required from the detector signal after each cycle. The purity of the products is confirmed by the chromatograms of each major fraction which are shown in figure 22. The chromatograms in figure 22 show that each fraction is very pure with very little isomer contamination. This relatively high purity was obtained, using a 400 mg charge, in less than 25 minutes. It is clear that the recycling procedure, coupled with the peak shaving technique, can handle significant column loads, provide the required resolution and at the same time provide excellent solvent economy.

Alternative Preparative Techniques

Although there may well be an economic limit to the scale of preparative chromatography, there does not appear to be any practical constraint to column size. Performance data from columns of over a meter in diameter have been reported which has shown that they can be operated very successfully. Unfortunately there is very limited design and operational data available for such columns due to the highly proprietary nature of their use. Large diameter columns, however, must have an appropriate sample distribution system at the column inlet. This is to ensure that the sample is dispersed evenly over the whole cross section of the column so that the full capacity of the column is utilized and as a secondary consideration significant column efficiency is not lost. Although the actual column may need to be custom made, solvent pumps, with high volume delivery at moderate pressures, are readily available. Most of the pipe fittings, valves, etc. can be obtained from manufacturers that supply equipment for general chemical plant operation. Large columns, handling large sample loads, will be effected by the heat generated in the column during solute absorption in the stationary phase. Consequently, it may require appropriate heat exchange systems to be arranged to prevent the separation being denigrated by localized temperature changes.

A number of alternative LC distributions systems (distribution systems, that is, other than simple columns) have been developed for preparative work and need to be described. Some of these have found limited use

but, nevertheless, have been shown to be very effective for large scale chromatographic purification for certain types of application. Unfortunately, the successful applications of these systems are also largely proprietary and so operating details are often not easy to obtain.

The Moving Bed Continuous Chromatography System

The idea of a continuous moving bed extraction process as a means of large scale separation is almost as old as gas chromatography itself. The first description of a continuous *gas-solid* extraction process that was reported in the chromatography literature was in 1956 by Freund *et al.* (10). However, much of the experimental work had been reported considerably earlier but in the 'not readily available' Hungarian technical literature. Shortly afterwards, the use of a moving bed for the continuous preparative scale product isolation using *gas-liquid* chromatography was reported by Scott (11,12)

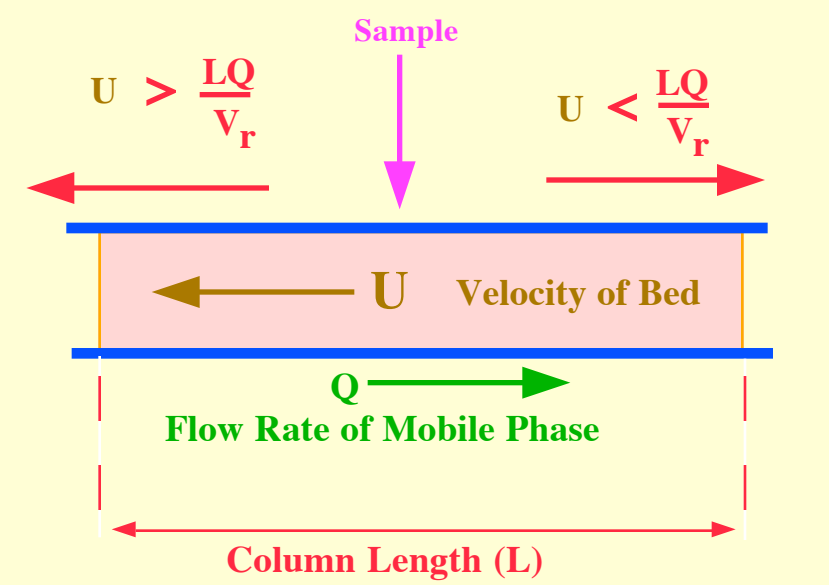


Figure 23. Diagrammatic Representation of a Moving Bed Separation System

The principle of the moving bed separating system is basically simple, but can be quite difficult to carry out in practice. Considering the separation from a theoretical aspect, the moving bed is depicted, diagramatically in figure 23. Let the column have a length (L) and *with the packing static* let the retention volume and retention time of a solute eluted through the column be (V_r) *ml*, and (t_o) *sec*. *respectively*. When

moving through the column from right to left, let the packing have a velocity (U). Let the mobile phase pass through the column from left to right at a flow rate (Q) ml / min..

$$\text{Now,} \qquad t_r = \frac{V_r}{Q}$$

The velocity of the band along the column (v) will be the ratio of the column length (L) to the retention time (t_r)

$$\text{Thus,} \qquad v = \frac{L}{t_r} = \frac{LQ}{V_r}$$

Now when $\frac{LQ}{V_r} > U$ then the solute will move through the column *in the direction of the mobile phase and be eluted in the mobile phase at the end of the column*.

And when $\frac{LQ}{V_r} < U$ then the solute will move through the column *in the opposite direction to the mobile phase and be eluted by the moving stationary phase at the beginning of the column*.

Consequently, if there are two solutes (S_1) and (S_2) which are continuously fed on to the center of the column, having retention volumes $V_{r(1)}$ and $V_{r(2)}$, respectively,

$$\text{then if} \qquad \left[\frac{LQ}{V_{r(1)}} < U\right] \text{ and } \left[\frac{LQ}{V_{r(2)}} > U\right]$$

one solute (S_2) will be eluted with the mobile phase at the end of the column and the other (S_1) will be eluted with stationary phase and support at the front of the column.The basic principles given above will

apply to all moving bed or simulated moving bed systems irrespective of the complex nature of the experimental design.

The Continuous Moving Process for the Isolation of Pure Acetylene Using Gas-Solid Chromatography

The moving bed process described in 1956 by Freund *et al*. (10) was developed to isolate pure acetylene from the gaseous product obtained from methane oxidation. The actual feed mixture contained 8-9% of acetylene, 4-5% of carbon dioxide, 4-5% of methane, 25% of carbon monoxide, and about 50% of hydrogen.

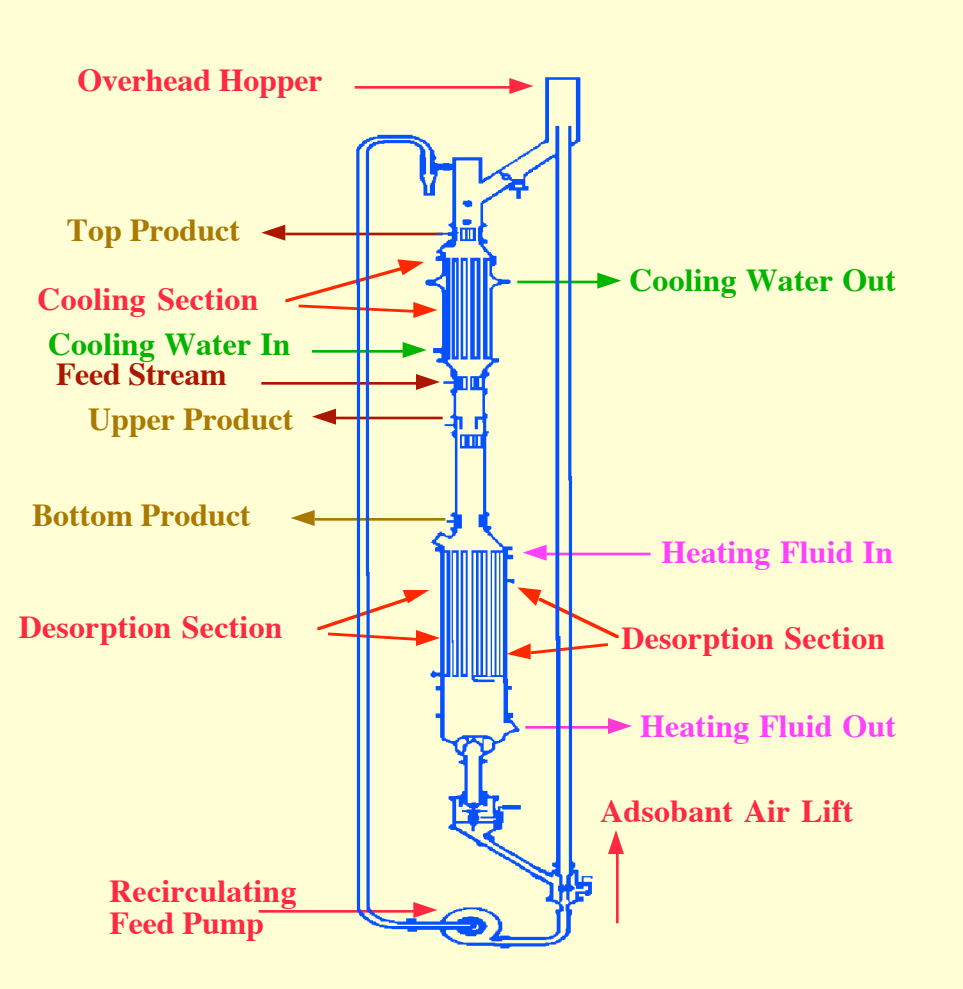


Figure 24. Apparatus for the Extraction of Pure Acetylene from a Gaseous Hydrocarbon Mixture By Continuous Adsorption Chromatography

The apparatus shown in figure 24 produces relatively pure acetylene as a direct product. The upper section is cooled with water to reduce the temperature of the carbon and allow the strong adsorption of acetylene, which takes place in the second section. The lighter gases are eluted at the top of the adsorber, (the top product). The cooled absorbent containing acetylene and carbon dioxide is heated and the carbon dioxide is first desorbed and collected as the upper product. As the adsorbent moves into a hotter part of the stripper section, the acetylene is desorbed and collected as the lower product. The product had a purity of about 98%.

Considering the very limited chromatography knowledge that was available in 1956, together with the limitations of the equipment that was accessible, the process and plant design was a very impressive achievement at that time.

The Continuous Moving Bed Process for the Isolation of Pure Benzene from Coal Gas

The moving bed technique was first applied to *gas-liquid* chromatography by Scott (11,12). A diagram of the moving bed system suitable for gas-liquid chromatography (GLC) was proposed by Scott also in 1956, and the basic system is depicted in figure 25. The original GC system will be used here to explain the principal of the separating process.

The procedure was used to isolate pure benzene from coal gas. Domestic gas at that time was derived from the pyrolysis of coal during the production of coke and contained a number of aromatic hydrocarbons including significant quantities of benzene and toluene and some xylenes. The stationary phase coated on a support is arranged to free-fall down a tower. This, in effect, provides the moving stationary phase. A gas feed containing the solute vapors enters the tower at the center. By the careful adjustment of pressures the gas is arranged to pass up the tower and out through an exit at the top. If the band velocity of a solute is greater than the velocity of stationary phase descent, then the solute will pass up the tower. If the solute velocity is less than the velocity of

stationary phase descent, then it will be carried down the tower in the moving stationary phase into the lower part of the tower.

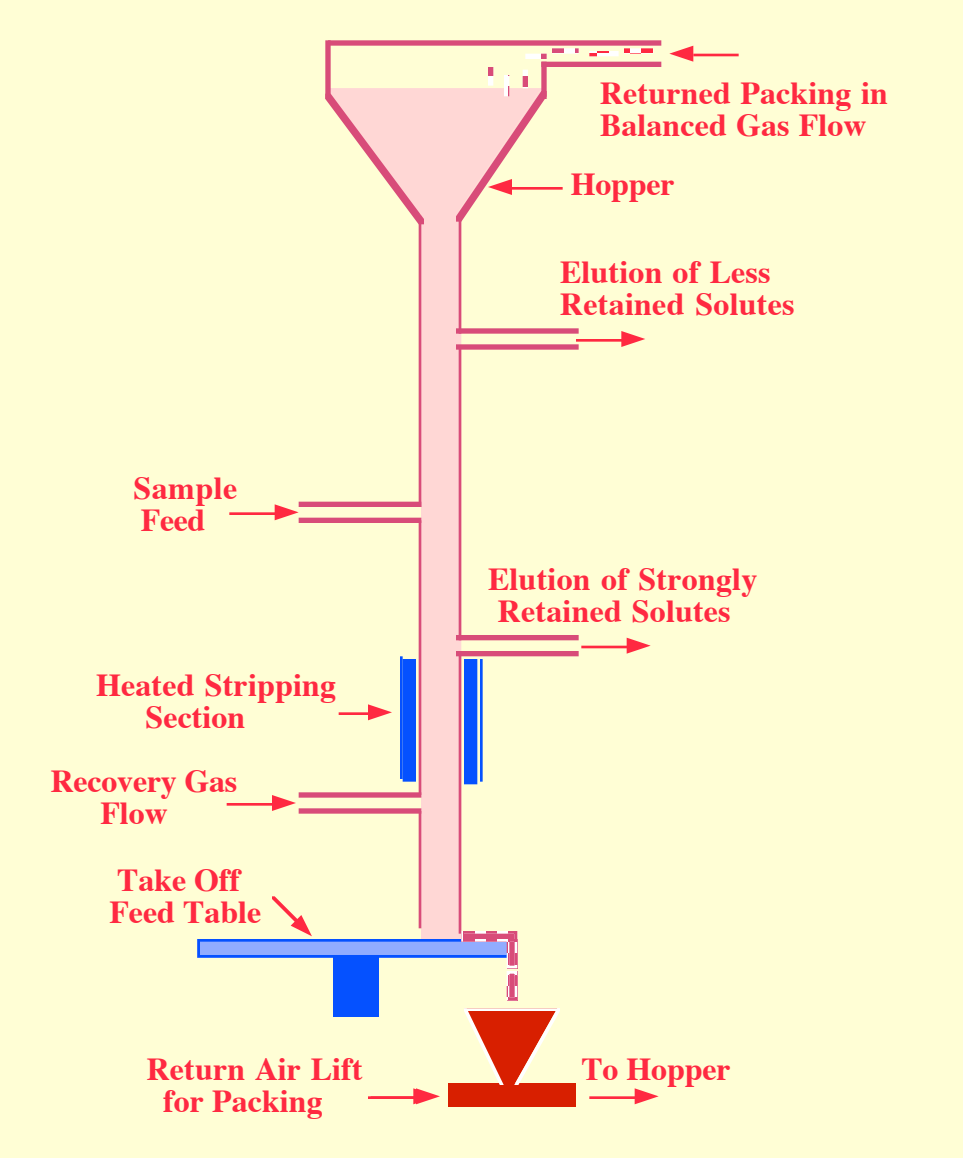
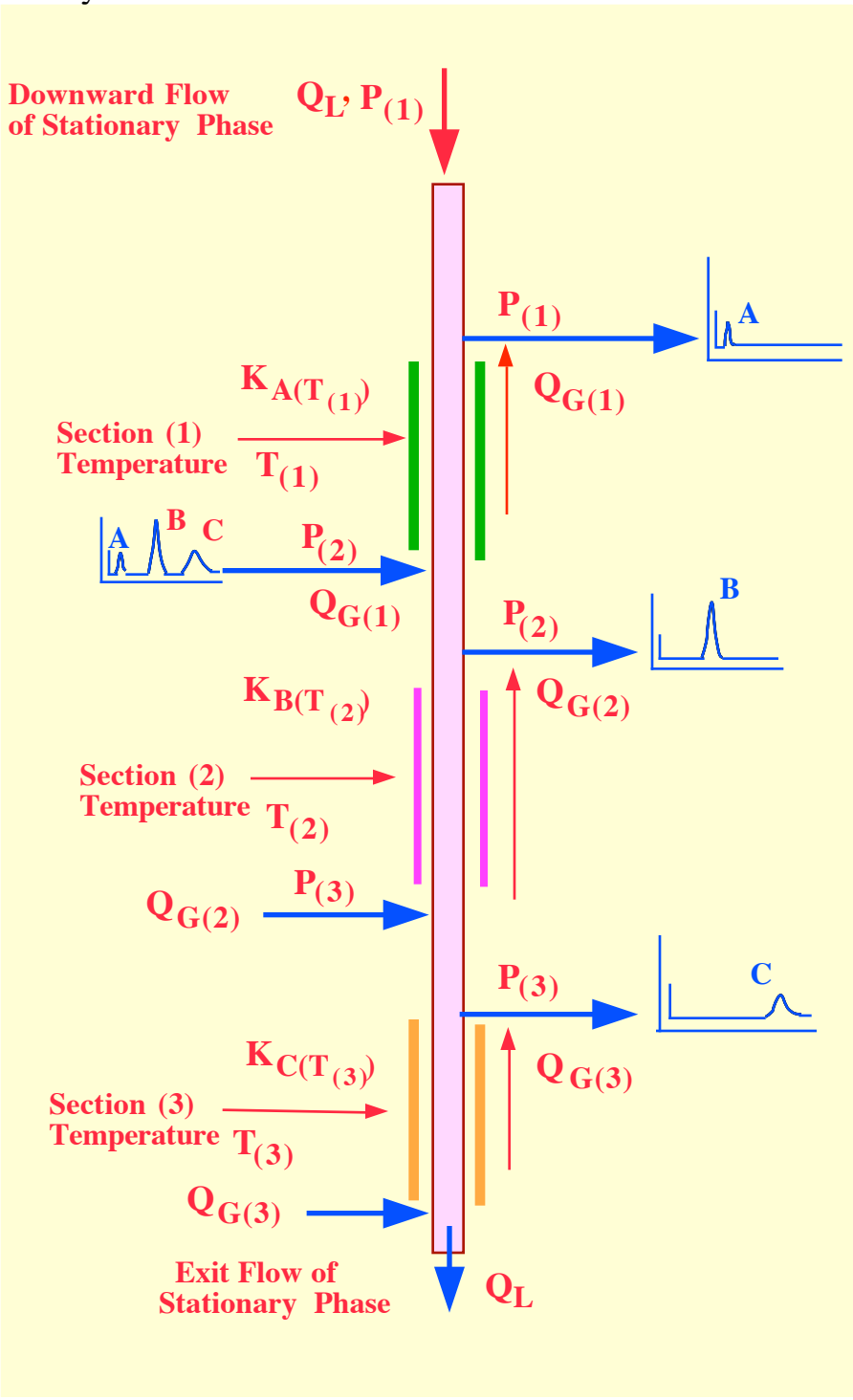


Figure 25. A Continuous Chromatography Separation System

The stationary phase in the lower part of the tower meets a second upward stream of gas and a portion of the lower part of the tower is heated. This results in the distribution coefficients of the solutes being reduced and their velocity up the tower increased so that they are now greater than the velocity of stationary phase descent. The stripping gas exits at another port at the top of the lower section of the tower. The

packing passes out of the tower at the base, the amount of fall being controlled by the rate of rotation of a feed Table.



Courtesy of Butterworths Scientific Publications Ltd. [Ref. 10]

Figure 26 The Moving Bed Continuous Gas-Liquid Chromatography System for Separating Pure Benzene from Coal Gas

The packing is then gas-lifted back to the top hopper for recycling. This procedure will split the solutes into two fractions and if the lower part of the tower contains a similar splitting system, then a specific solute can be selected from the mixture and obtained pure.

The practical system used by Scott for the isolation of pure benzene from coal gas is shown diagramatically in figure 26. Let the gas flow carrying the sample through the upper half of the tower be ($Q_{G(1)}$). Let the volume of mobile phase passing down the tower per unit time be (Q_L) and the distribution of solute (A) between the stationary phase and the mobile phase at temperature ($T_{(1)}$) be ($K_{A(T)}$).

Assuming the down velocity of the stationary phase is (Q_L), and that the retention volume (V'_r) is given by $V'_r = KV_L$ (see the Plate Theory Book 6) then if

$$\frac{Q_{(G)}}{K_{(A)}V_L} > Q_L$$

then solute (A) will pass up the column.

Conversely, if $$\frac{Q_{(G)}}{K_{(A)}V_L} < Q_L$$

then solute (A) will pass down the column.
The conditions for the continuous separation of substances (A), (B) and (C) are now clear.

In section (1), solute (A) will be separated from solutes (B) and (C) when,

$$\frac{Q_{G(1)}}{K_{A(T_{(1)})}V_L} > Q_L \qquad (3)$$

$$\frac{Q_{G(2)}}{K_{B(T_{(2)})}V_L} < Q_L \qquad (4)$$

$$\frac{Q_{G(3)}}{K_{C(T_{(3)})}V_L} < Q_L \qquad (5)$$

These conditions will ensure that solute (A) passes up the tower and solutes (B) and (C) pass down the tower.

Thus, the flow of stationary phase and support down the column carrying the sample into section (1) must be regulated so that at temperature ($T_{(1)}$) the conditions defined in equations (3), (4) and (5) are met. In addition by employing suitable restrictions, the pressure at the top of the tower must be made equal or close to that of the outlet for solute (A) to prevent cross-flow.

Now to separate solute (B) form (C) the following conditions must be met,

$$\frac{Q_{G(2)}}{K_{B(T_{(2)})} V_L} > Q_L \qquad (6)$$

and

$$\frac{Q_{G(3)}}{K_{C(T_{(3)})} V_L} < Q_L \qquad (7)$$

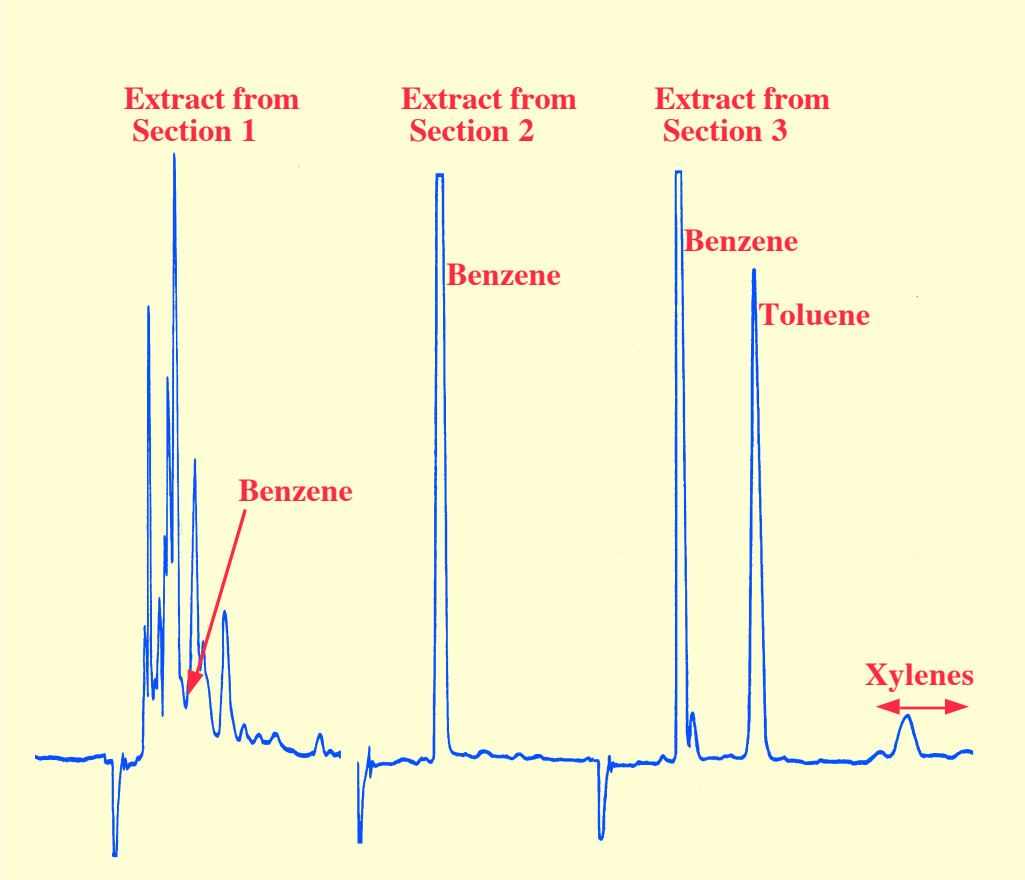
Having established the down-flow of stationary phase the conditions defined in equations (6) and (7) must be met by adjustment of ($Q_{G(2)}$) and ($T_{(2)}$). In a similar manner, using suitable restrictions, the pressures at the sample inlet and that of the outlet for solute (B) must be made equal (or close to equal) to reduce cross-flow.

Finally, solute (C) can be recovered under the following condition,

$$\frac{Q_{G(3)}}{K_{C(T_{(3)})} V_L} > Q_L \qquad (8)$$

By adjusting the magnitudes of ($Q_{G(3)}$) and ($T_{(3)}$), the conditions defined by equation (8) can be established. As would be expected, to prevent cross-flow, the pressures at the second stripping flow inlet and outlet for solute (C) must also be made equal, or close to equal. Scott and Maggs (12) designed a three stage moving bed system, physically very similar to that described above, to extract pure benzene from coal

gas. Coal gas contains a range of saturated aliphatic hydrocarbons, alkenes, naphthenes and aromatics. In the above discussion the saturated aliphatic hydrocarbons, alkenes and naphthenes are represented by solute (A).



Courtesy of Benzole Producers Ltd. (ref.7)

Figure 27 The Extraction of Pure Benzene from Coal Gas by continuous Extraction Using a Moving Bed Technique

The main product, benzene, is represented by solute (B), and the high boiling aromatics are represented by solute (C) (toluene and xylenes). An analysis of the products that were obtained are shown in figure 27. The material stripped form the top (section (1)) is seen to contain the alkanes, alkenes and naphthenes and *very little benzene*. The product stripped from the center section appears to be virtually pure benzene. The product from section (3) contained toluene, the xylenes and thiophen which elutes close to benzene. The thiophen, however, was only

eliminated at the expense of some loss of benzene to the lower stripping section. Although the system worked well, Scott and Maggs found it experimentally difficult to set up and maintain production under constant operating conditions. The problems arose largely from the need to adjust the pressures that must prevent cross-flow. Without doubt, today, the system would be computer controlled. The simple system as described, although satisfactory for GC operation, would be virtually impossible to operate with a liquid mobile phase.

The Simulated Moving Bed Preparative Chromatography System

The physical system described above was found to be extremely tricky to operate (although with modern computer control technology, the difficulties may well be significantly reduced) and this stimulated research into alternative moving bed systems. In 1971, Barker (13) and in 1973 Barker and Deeble (14) used a column in circular form to imitate the falling bed system. A diagram representing the wheel concept of Barker and Deeble is shown in figure 28.

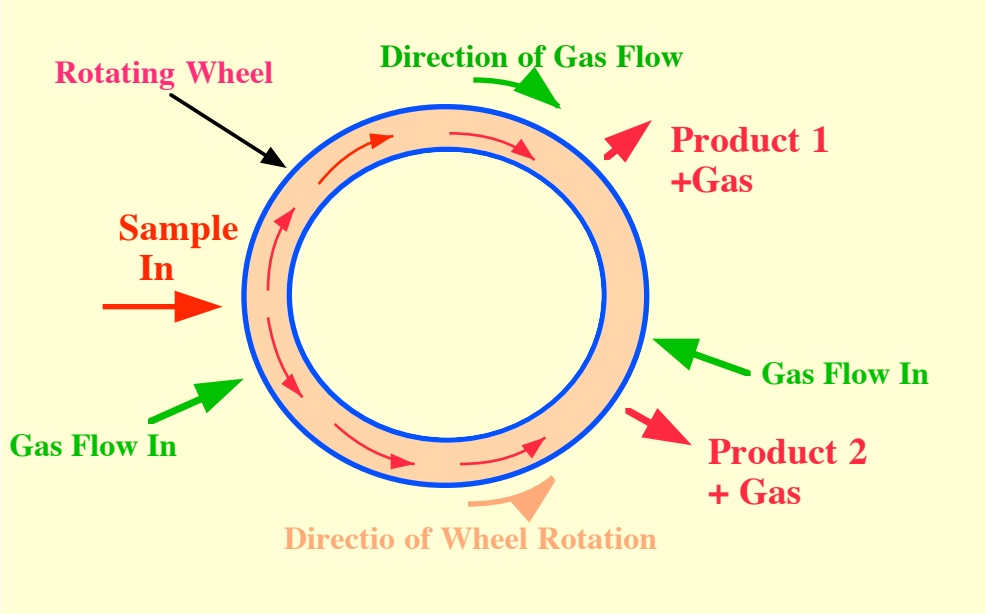


Figure 28 The Wheel Concept of Barker

The actual column consisted of a peripheral groove in a large wheel in which the packing is placed and contained by a plate placed on the

outside after packing. The wheel somewhat resembled the driving wheel of a medium sized steam locomotive.

The ports to the column are fixed in positions at the periphery of the large wheel and the wheel had openings fitted with 'pop-valves regularly round the circumference to allow the ports to be continually connected to the peripheral column as it was rotated. The peripheral apertures were designed so that they were normally closed except when they coincided with the ports during rotation.

In this way, the bed moved continuously in one direction relative to the ports and the mobile phase moved in the opposite direction. As with the moving bed system, some solutes (those that eluted at a rate slower than the wheel velocity) moved in the direction of the wheel movement while others (those that migrated faster than the wheel velocity) moved in the opposite direction with the movement of the mobile phase.

The two fractions were collected from 'take-off' ports. This was the first form of the so called *simulated moving bed system,* and was used by Barker for continuous GC separations with moderate success. The main problems met in this system were leaks that occurred at the port seals on the wheel periphery, which seriously, limited the pressure that could be applied to the system to produce the mobile phase flow. The wheel was about 5 ft in diameter and thus was a very heavy and rather cumbersome device.

The first practical *simulated* moving bed system was described by Hurrel in the late 1960s (15). The Hurrel system was basically an ingenious extension of the large circular column devised by Barker. He employed a number of short preparative columns connected in series and rotation was simulated by mean of a somewhat complex set of valves mounted in the form of a disc. This procedure had two main advantages over the *actual* moving bed system. In addition to the technical and constructional simplicity, there is considerable adsorbent conservation and, as the mobile phase is recirculated, there is substantial solvent economy. The system devised by Hurrel is shown diagramatically in figure 29.

The circular column consists of a number of sections, each section taking the form of a relatively short preparative column. The columns are formed in the shape of a slanting U to facilitate packing, and are packed individually employing standard packing techniques. The columns are all joined in series by means of the large rotary disc valve fitted with appropriate ports. The disc valve could be fabricated in a relatively simple manner, compared with the construction of the massive rotating wheel device and, by suitable surface grinding and lapping, could be made leak proof even at relatively high pressures.

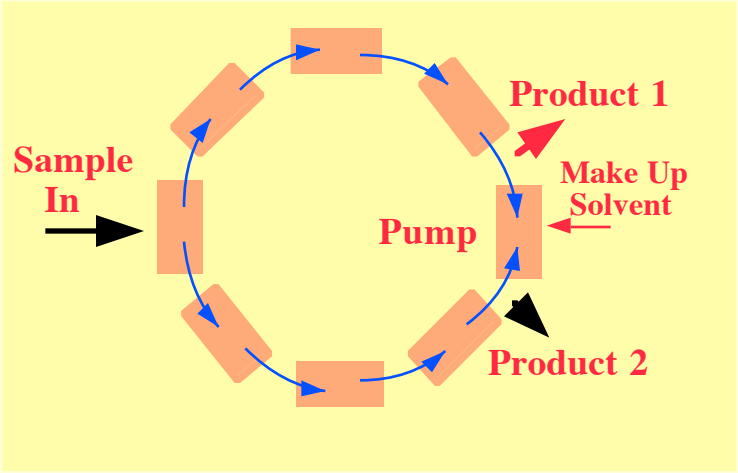


Figure 29. The Multi-Column Simulated Moving Bed Column

The disc was constructed from two thick steel discs, the lower disc carried the connections to the columns and the upper disc carried the connections to the different inlet and outlet ports. The lower disc was assembled on a steel framework to provide support for the columns. The upper disc, was thus free to rotate and, when programmed correctly, could simulate the moving bed.

The disc was fairly complex in design and contained many sliding surfaces all of which had to be leak proof when under considerable external pressure. Nevertheless, it could be constructed relatively simply when compared with the complex construction of Barkers large wheel device. Using appropriate surface grinders and lapping procedures, despite its size the disc valve could be made leak proof even when under hydraulic pressure when used for liquid chromatography separations.

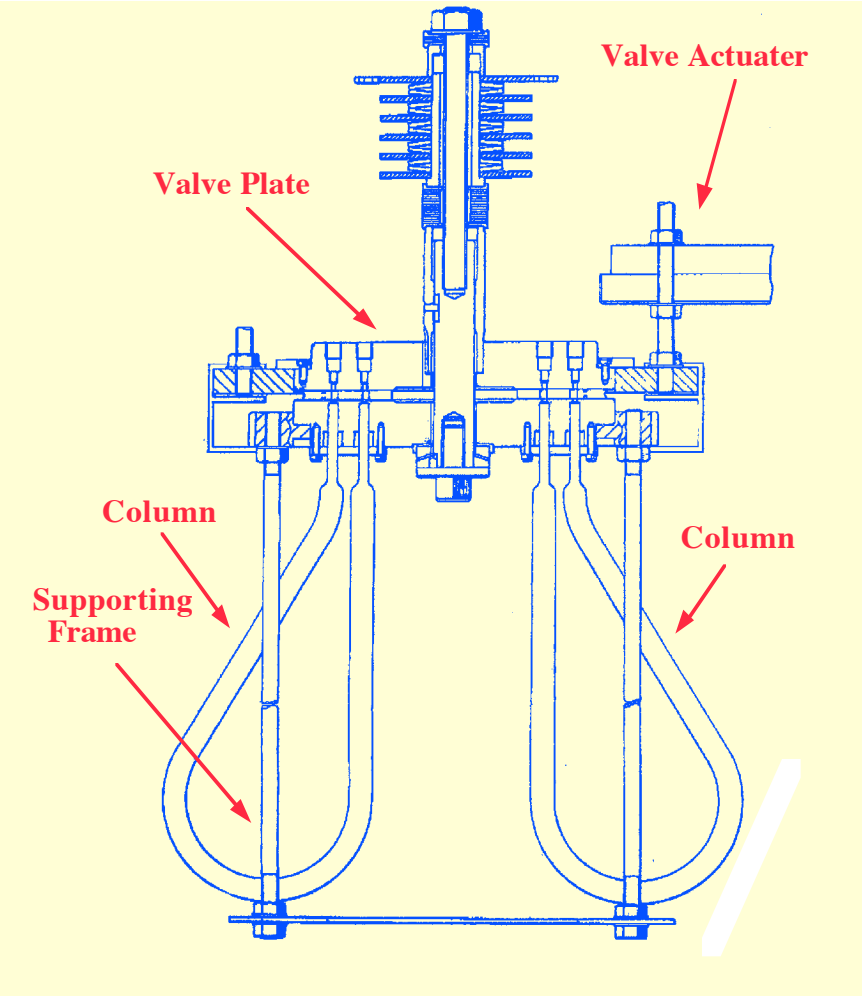


Figure 30. A Cross section Of the Hurrel Disc Valve System and Columns.

A diagram of the cross-section of a valve including attached columns (the columns can be constructed of glass or metal depending on whether the system is to be used for GC or LC) is shown in figure 30. They are packed using standard packing procedures similar to those previously discussed. The design of the columns and column-disc connection allow easy column replacement if columns become damaged, contaminated or the stationary phase changed. The oblique shape of the columns allow the overall system to be relatively compact and, at the same time, have a reasonably high loading capacity.

Contemporary simulated moving bed systems that are used for preparative chromatography are mostly based on the Hurrel concept They all work basically on the same principle and the simple theory given for an actual moving bed devices will apply, albeit in a somewhat modified form. In modern simulated moving bed systems, the valving methodology has been developed further and is more complex. More recent systems are depicted somewhat differently as shown in figure 31 and can be oriented in a number of different ways, although they are all basically derivatives of the Hurrel system.

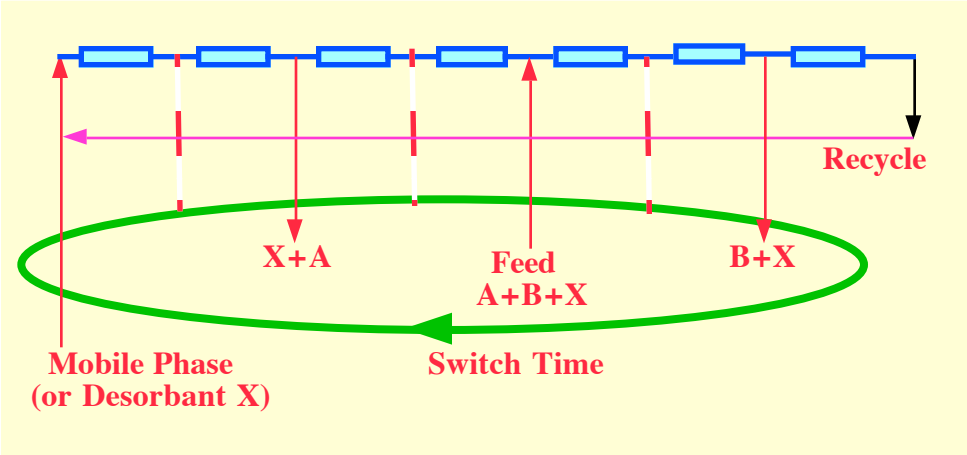


Figure 31. The Modern form of Depicting Simulated Moving Bed Technology

The mobile phase passes through several stationary columns which contain the stationary phase which may be silica gel, or a bonded phase, or, in GC a suitable coated support. There are, also, a number of different ports, one for the mobile phase and one for the return of the mobile phase. In addition there is a central feed port and two take-off ports. These ports can, by appropriate valve programming (usually by means of a computer) connect, sequentially, each column to its neighbor. In modern simulated moving bed systems, disc valves are no longer employed and have been replaced by sets of individual valves. It is clear that the apparent counter current movement of the stationary phase, relative to the mobile phase, is achieved by appropriate valve switching which simulates the rotation of the ports on the disc valve between each column.

Referring to figure 31, it is seen that part of the feed moves with the mobile phase and is collected by a small take-off flow in front of the feed port (B + solvent). The other, more retained portion of the sample, accumulates in a column on the other side of the feed port and is collected by a another small take-off flow behind the feed port (A + solvent). This particular system ideally, produces two products and thus could lend itself specifically to the separation of enantiomeric pairs. However, for effective separation with high purity yields, the stationary phase capacity for the two enantiomers must be fairly large and thus the phase system must be carefully selected. The technique has been successfully used to isolate single enantiomer drugs (16-18).

Radial Flow Chromatography

Another alternative chromatographic procedure for preparative separations is *radial flow chromatography.* The radial flow chromatography column consist of two concentric porous cylindrical frits between which the stationary phase is packed. basically, it is only effective when the separation ratios between the substance of interest and its neighbors is fairly high. This is because the column itself has a very limited length (equivalent to the radial thickness of the packing) and thus can produce relatively few theoretical plates. The efficiency can be significantly improved by using very small particles but the effective use of the radial column also requires a very homogeneous packing. A diagram of a radial chromatography column is shown in figure 32.

The mobile phase flows from the outer cylindrical frit, across the radius of the column, through the cylindrical bed of stationary phase, to the inner cylindrical frit. The radial gap between the frits is the effective bed 'height.' or 'length'. The core houses the inner frit, through which the eluent percolates and exits at the base of the column to a detector and hence to a fraction collector. The outer frit constitutes the column inlet, and consequently the sample has initially an extremely large area of stationary phase with which to interact. This renders the loading capacity of the radial flow column also very high. It is interesting to note, that as the solute progress radially through the stationary phase bed towards the center, the effective cross-sectional area of the column will become

smaller. Consequently, the plate volume of the column will decrease (see Book 6) as the solute moves to the center which will result in the solute being concentrated.

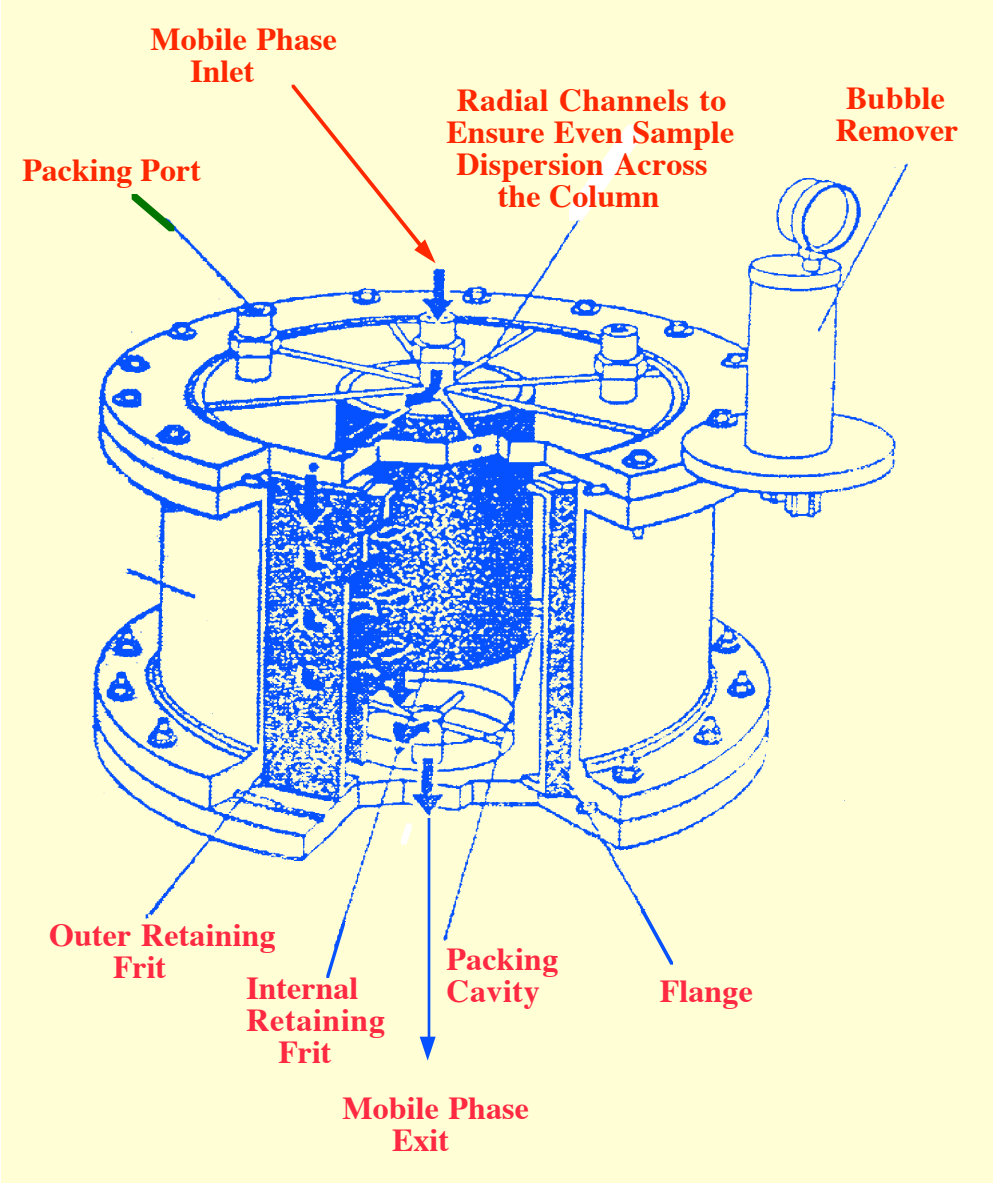


Figure 32. The Radial Flow Column

However, as the solute bands progressively decrease in concentration due to normal dispersion processes (see Book 9), this counteracts the concentration effect from reduced bed cross-section and prevents the column packing from becoming overloaded at the center.

The sample is injected on the top of the column, where it is dispersed by radial channels (see figure 32.) to ensure an even sample loading around

the periphery of the column. Although this type of column has a high capacity, it is also relatively short and thus not suitable for complex mixtures that need to be developed isocratically. They can, however, be used very effectively with gradient elution.

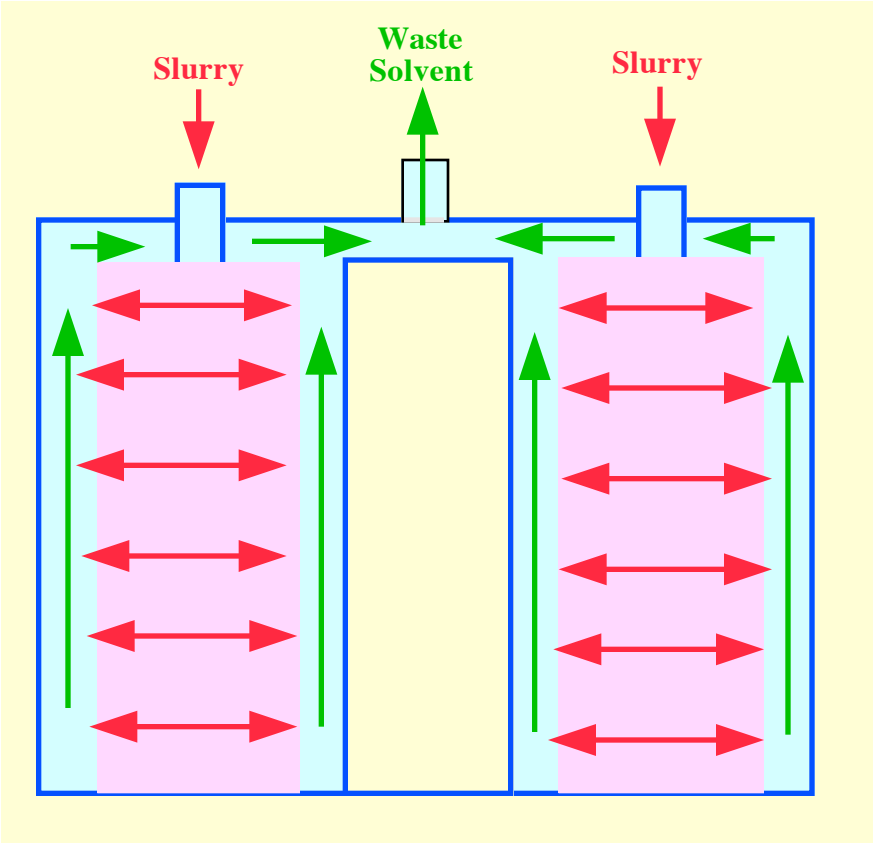


Figure 33. Packing Radial Columns

In practice, the gradient is made to 'lift' the solutes from the packing by the progressive increase in either the dispersive solvent component of the mobile phase, the polar component, salt concentration or the pH depending on the type of phase system selected. This means it is generally restricted to the separation of substances that have relatively large separation ratios and differ significantly in the strength of their interaction with the stationary phase. Due to the cross-sectional area decreasing as the solute progresses through the bed, the linear velocity of the mobile phase also changes and so the optimum velocity can only be a compromise for the total bed length. The method used for packing radial columns is depicted in figure 33. The packing is prepared in the form of a slurry and is pumped directly into the column between the two

frits. The column exit to the detector and fraction collector is closed, and, as a result, the slurry solvent passes through the outer frit and exits via the normal mobile phase inlet port. The columns are very easily unpacked by adopting the reverse procedure.

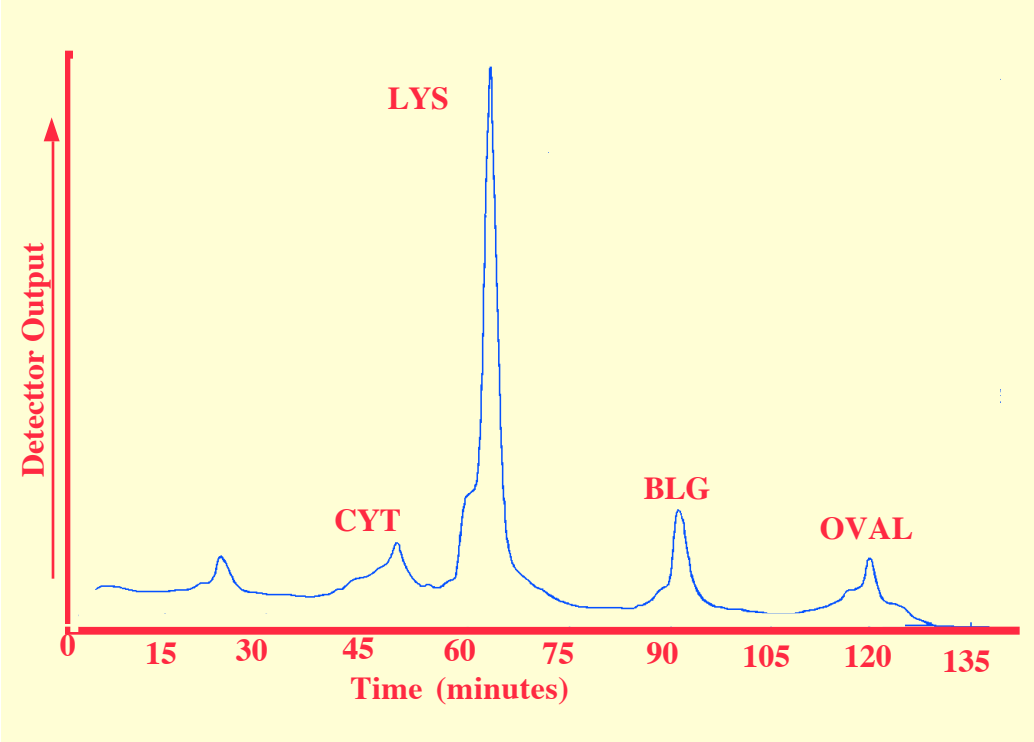


Figure 34. The Separation of Some Large Biomolecules Using Radial-Flow Reverse Phase Chromatography

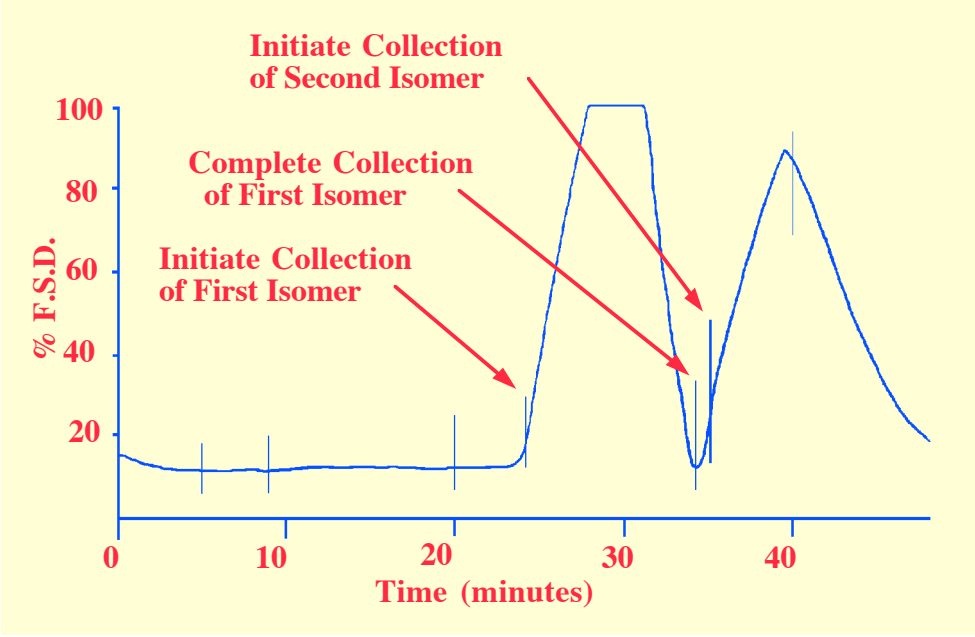
An example of the use of the radial flow column to separate some large biomolecules is shown in figure 34. The total column volume was 500 ml (that is the space between the cylindrical frits) which was filled with a proprietary packing, IMPAQ^{R} RG2020-C18 (a basically dispersive stationary phase using octadecane bonded to silica gel). The solutes were separated in the reversed phase mode. A linear gradient was employed starting with acetonitrile/0.1% TFA in water : 20/80 v/v, and terminating with acetonitrile/0.1% TFA in water : 50/50 v/v. The gradient period was 120 minutes and the flow rate was 46 ml per min.

The Preparative Separation of the Enantiomers of Chlorokynurenine

Details of this application were provided by ASTEC Inc. who obtained it by private communication from Dr. Lester Dolak and Dr. Eric Seest of

the Upjohn Pharmacia Company, Kalamazoo. The column that was employed was 50 cm x 5 cm (Chirobiotic T column from ASTEC) and the separation was carried out at 40˚C. It was a little difficult to arrive at a suitable feed solvent due to the relative insolubility of the solute in the mobile phase. It was eventually dissolved at a concentration of 2 mg per ml of chlorokynurenine in a solvent (95% aqueous ethanol/water : 50/50 v/v) by heating and stirring at 40˚C. The filtered sample solution and the column were maintained at 40˚C throughout the sampling procedure and separation. 400 ml of the solution (containing 800 mg of chlorokynurenine) were pumped onto the column at 50 ml/min. for 8 minutes. The sample pump was then stopped, the solvent pump started and the solutes eluted at a flow rate of 50 ml/min. for 20 minutes.

As soon as the second enantiomer began to emerge, the flow rate was increased to 60 ml/min. An actual separation is shown in figure 35. and it is seen that the separation that was obtained was highly satisfactory.



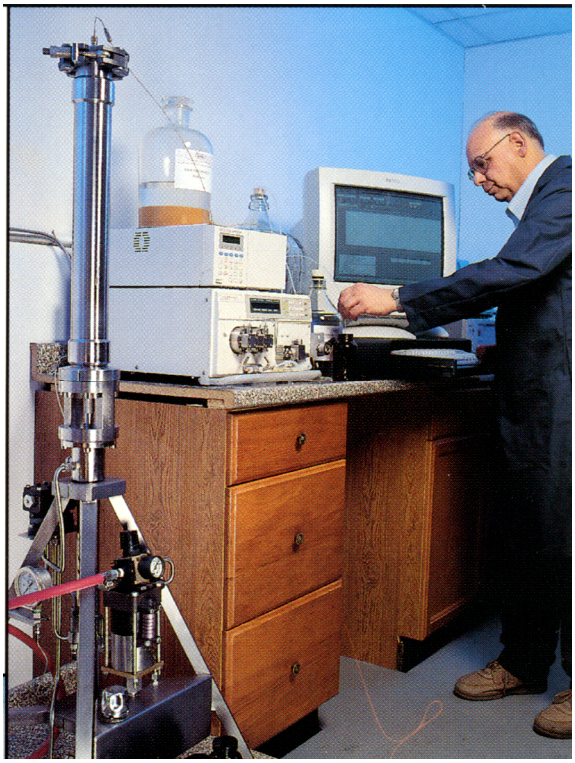
Courtesy of Dr. Lester Dolak and Dr Eric Seest of Pharmacia Upjohn Inc.

Figure 35. The Preparative Separation of the Enantiomers of Chlorokynurenine

The products were analyzed on an analytical Chirobiotic T column and indicated that the first enantiomer was >99% pure, and the second enantiomer was 98% pure. The mid fraction, that was collected between

the two main peaks, was recycled. The total cycle took 49 minutes and it is seen that the system operated very effectively. The use of preparative chromatography for the separation of physiologically active enantiomers is now quite well established but, even so, it is probably still in its infancy and many improvements are likely to occur in the future. The technique of LC is probably the only practical technique for the large scale resolution of many racemic mixtures.

An example of a commercially available preparative column designed for the separation of enantiomers is shown in figure 36. The column was manufactured by ASTEC Inc. who have a number of different types of chiral stationary phases available for preparative use. These columns vary in size from a few cm in length to a meter or more.



Courtesy of ASTEC Inc.

Figure 36. A Commercially Available Preparative Column for the Separation of Enantiomeric Pairs.

The separation of the enantiomers of chiral drugs has become exceedingly important over recent years. This new enthusiasm was fostered by the discovery that the respective physiological activity of the

isomers of a drug could differ radically and this was found to be true for many physiologically active compounds and, in particular, physiologically active biotechnology products. However, the major stimulation arose from the unfortunate birth defects initiated by one of the enantiomers of Thalidomide. This drug was manufactured and sold as a racemic mixture of N-phthalylglutamic acid imide. However, the desired physiologically activity was found to reside solely in the R-(+)-isomer and it was discovered, too late, that the corresponding S-(-)-enantiomer was teratogenic and caused serious fetal malformations. Enantiomers of a single substance are essentially chemically identical and only differ in the spatial arrangement of chemical groups or atoms around a single atom, referred to as the stereogenic center or the chiral center(s). Such differences impart very subtle physical chemical variations between the individual isomers and, thus, must be carefully exploited if a chromatographic separation of the individual enantiomers is to be achieved. A number of antibiotics have been shown to be very effective stationary phases for the separation of chiral mixtures. An example of one of these is Vancomycin the structure of which is depicted in figure 37. Vancomycin has three fused rings (shown as rings A, B and C in figure 37) and 18 chiral centers which endows it with the characteristic strong chiral selectivity exhibited by many antibiotics.

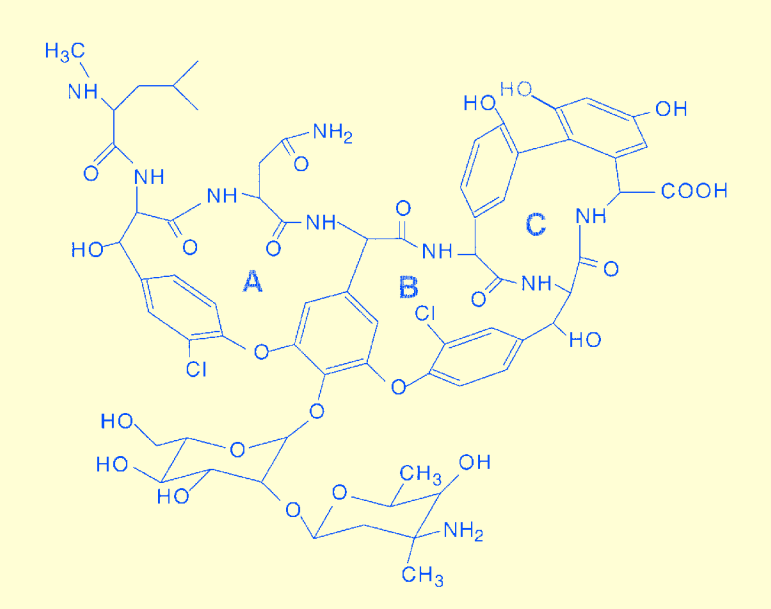
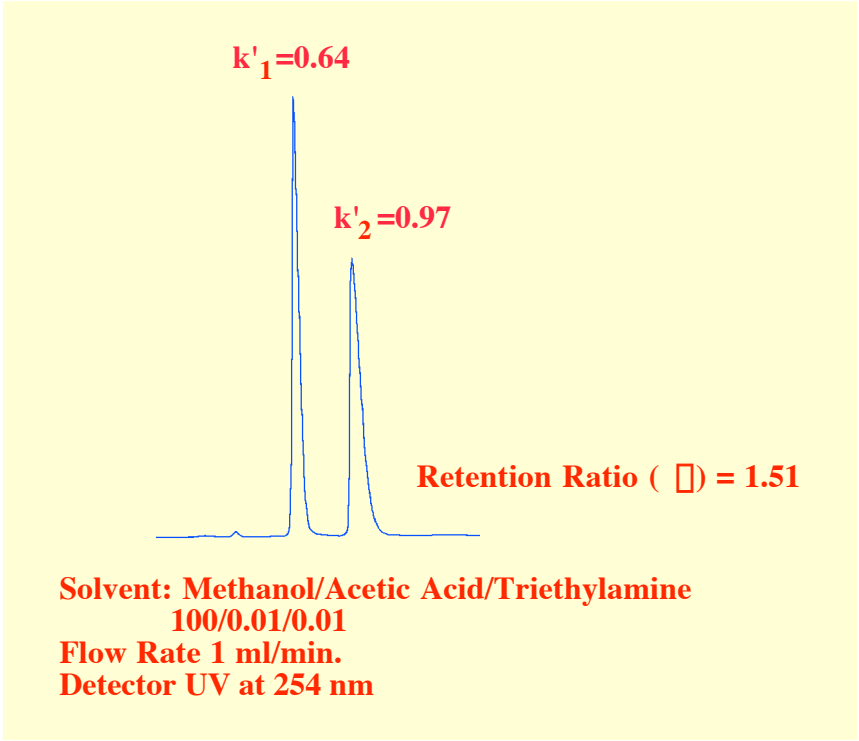


Figure 37. The Structure of Vancomycin

It also contains two sugar moieties and has a molecular weight of 1449. This material has multimodal capabilities, great stability and high chromatographic capacity for preparative work. It is complimentary in chiral selectivity to the antibiotics Teicoplanin and Ristocetin. An example of the use of Vancomycin in preparative chromatography is demonstrated in the separation of the enantiomers of Nicardipine. Nicardipine is a cerebral and coronary dilator with calcium blocking activity. It chemical name is 1,4-dihydro2,6-dimethyl4-[3-nitrophenol]3,5-pyridine dicarboxylic methyl2-[methyl (phenylmethyl)amino]ester. It exists in two isomeric forms the (□) form M.Pt.168-173˚C and the (□) form M.Pt. 179-181˚C.

The chromatographic properties of the enantiomers of nicardipine on the phase system selected for their preparative separation is shown in figure 38.

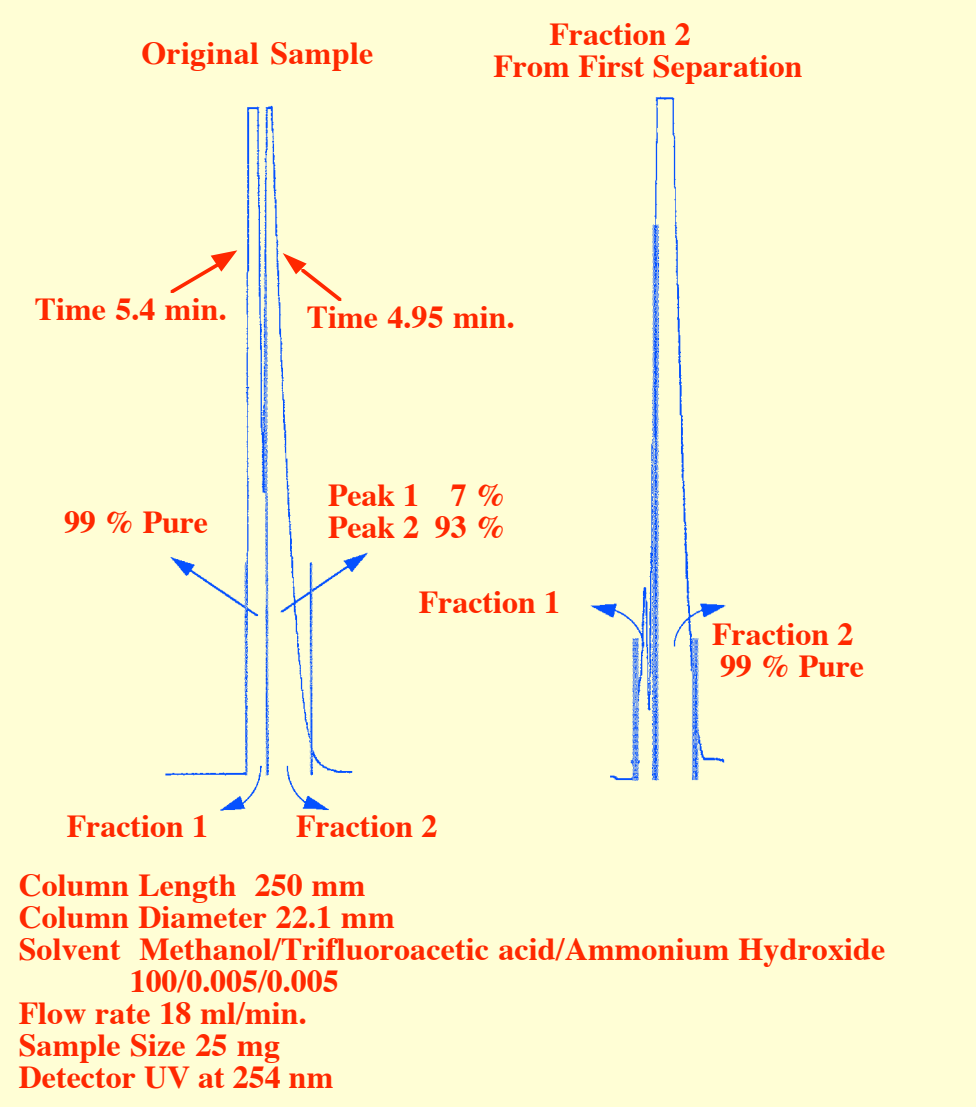


Courtesy of ASTEC Inc.

Figure 38. The Chromatographic Properties of the Enantiomers of Nicardipine when Retained on the Chirobiotic Vancomycin

It is seen that the solvent system selected elutes both isomers at fairly low capacity ratios (*cf.* 0.64 and 0.97) but the separation ratio is still

relatively high (*cf.* 1.51) which will allow considerable column overload. These advantageous retentive properties are afforded by he unique character of chirobiotic stationary phase. The mobile phase (termed by the manufacturers as elution by the *polar organic mode*) is strongly polar so the major retentive mechanism is likely to be predominantly dispersive in nature modified by the selective interactions afforded by the chiral centers of the Vancomycin (see book 7 of this series).



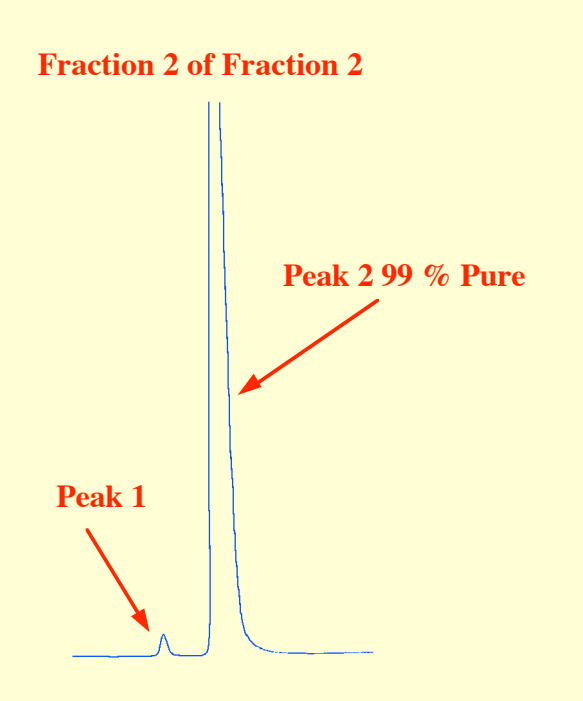
Courtesy of ASTEC Inc.

Figure 39. The Primary Preparative Separation of the Isomers of Nicardipine.

The preparative column that was employed was 25 cm long, 2.21 cm in diameter and operated at a flow rate of 18 ml/min. The mobile phase (the

polar organic solvent) was a mixture of methanol, trifluoroacetic acid and ammonium hydroxide (100/0.005/0.005). The initial sample size was 25 mg and the separation obtained is shown in figure 39.

The first fraction was taken between the beginning of the first peak and the minimum between the peaks. As the peak were somewhat asymmetrical due to the column overload the first fraction was 100 % pure peak 1. The second fraction (mostly peak 2), however, was significantly contaminated with peak 1. The second fraction was then run again, under exactly the sample conditions. Now, however, the first peak is not overloaded and is thus symmetrical. The second peak, however, is still overloaded and asymmetrical but will have a sharp front. Consequently if the second fraction is taken between the minimum between peak 1 and peak 2 and the end of peak 2 the product will be almost pure peak 2.



Courtesy of ASTEC Inc.

Figure 40. The Second Preparative Separation of the Isomers of Nicardipine

The first fraction of the second separation is bulked with the original mixture and recycled. An analytical separation of fraction 2 of the

second separation is shown in figure 40. The separation was carried out under the same conditions as those shown in the separation in figure 38. It is seen that the second enantiomer is also obtained in a state of high purity and furthermore because the third fraction is recycled there is little loss of material

A summary of the purities and yields of the two isomers is shown in Table 2. It is clear that although the separation involves the resolution of two chemically very similar compounds, tens of milligrams of material could be separated in relatively high states of purity employing a column only 25 cm long and less that an inch in diameter.

Table 2. Summary of the Purity and Yield from the Separation of the Isomers of Nicardipine

Final Results	Peak 1	Peak 2
Purity	99 %	99 %
Yield	95%	90 %

It should be emphasized that the success of this preparative separation depended, not on high column efficiencies, or, for that matter, on a particularly large column, but on the selection of a phase system that provided a high retention ratio between the two isomers. The required selectivity was achieved by firstly choosing a stationary phase with strong chiral interactive properties and secondly by choosing the best complementary solvent system that would provide strong differential interaction between the isomers and the two phases.

Criteria for the Successful Operation of Preparative Chromatographs.

Preparative chromatography, or perhaps one should say *semi*-preparative chromatography, is not difficult to carry out providing some basic requirements are met most, of which have already been described in some detail. The following is a summary of the essential requisites for a successful preparative separations.

1. The stationary phase and the combined phase system must be very carefully chosen to provide the maximum separation between the solute of interest and its nearest neighbor.

The mechanism of retention has been discussed in detail in book 7 of this series and reference should be made to the appropriate sections of that book. In general, the interactive character of the solute to be separated must be carefully considered, and the stationary phase chosen to have the appropriate interacting moieties that will enhance its interaction with the primary substance relative to that of the neighboring substance or impurity. In contrast the mobile phase will be chosen to have a quite different interactive character so that selectivity for the substance of interest will remain in the stationary phase. As a simple example, if the solute of interest is strongly polar, and the neighboring substance dispersive, then the stationary phase must be polar (*e.g.*, silica gel in LC, or perhaps, polyethylene glycol in GC) and in LC the mobile phase would be made predominantly dispersive (*e.g.*, *n*-heptane or methylene dichloride or mixtures of both) so that polar selectivity remains dominant in the stationary phase. Unfortunately, all substances are not simply polar or dispersive or ionic but can exhibit various combinations of all three interactive characteristics. It follows, that the stationary phase will also need to have the appropriate mixture of interactive properties to maximize selectivity and in contrast the interactive character of the mobile phase will need to oppositely balanced to ensure maximum selectivity still resides in the stationary phase. Under some circumstances the selectivity of the mobile phase can also be exploited to interact more strongly with the neighboring substance and thus elute it more quickly. It is clear, that some considerable effort must be made to identify the bests phase system for the isolation of a substance from a hitherto unknown mixture. This work can be carried out on an analytical column and its success will determine the maximum load that can be used, the purity of the products and time taken for the separation.

2. It is far more effective, less expensive, and in most cases faster, to over load a column to the maximum possible level and run a repeat series of separations, than to attempt to prepare large columns to handle the same sample in a single run.

The use of column over load has been discussed in detail in the early chapters of this book. Mass over load is to be preferred to volume over load, although the latter may well be preferable, if the solutes of interest

have a limited solubility in the mobile phase that provides the optimum selectivity. Mass overload also produces asymmetric peaks with a sharp front and a sloping tail which can be advantageous for the production of high purity fractions by peak cutting. This advantage is illustrated in the last example given. The first peak can be collected as a fraction up to a point just before the sharp front of the second peak starts. This will produce a very pure fraction of the first peak The second peak is re-run in an identical manner and as the first peak is now an impurity (and therefore present at a low concentration) it is not overloaded and, therefore, will be eluted as a symmetrical peak in front of the second main peak. If required, the second peak can be collected just after the trace of the first peak is eluted and will also be extremely pure. The first fraction of the second separation, still containing a mixture of both peaks, although containing a very small percentage of the total mixture can be recycled if considered appropriate.

3. If large sample loops are employed the injection must be cut so that tail of sample left in the loop does not cause serious peak dispersion and loss of resolution

Sample cutting is a common technique in preparative separations and, as shown by the example earlier in the chapter, can eliminate significant peak dispersion which can result in either low product purity or reduced yield.

4. Under certain circumstances the column dimensions can be increases to improve both load and product yield.

There are two ways of increasing the size of the preparative column, by increasing its length and by increasing its diameter. Increasing the diameter increases the loading capacity and maintains the same separation time. It does not, however, increase the resolution. Increasing the column length increases the separation time and the resolution but does not increase the loading capacity, if the maximum efficiency is needed. If the sample consists of a simple pair of substances (*e.g.*, the separation of a pair of enantiomers) then increasing the column length will allow multiple samples to be separated in the column at one time

and, thus, will increase throughput and effectively reduces the separation time.

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